# SPRINKLER STORAGE OF WINDTHROWN PINUS RADIATA AT BALMORAL, NEW ZEALAND

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A thesis

presented for the degree

of

Doctor of Philosophy in Forestry

in the

University of Canterbury

Christchurch, New Zealand

by

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1981

'The truth is that as a man's real power grows and his knowledge widens, ever the way he can follow grows narrower: until at last he chooses nothing, but does only and wholly what he must do.....'

Ursula Le Guin 1968

# A B S T R A C T

Sprinkling of 43,000$m^3$ of windthrown *Pinus radiata* sawlogs was carried out at Balmoral State Forest, New Zealand from 1976 to 1980. This project investigated the microbial flora which had developed after three and four years sprinkling and determined the change to wood microstructure and related properties. Earlier stages of microbial colonisation were studied in an experimental logpile of *P. radiata*.

After four years sprinkling static bending tests showed an increase in the modulus of rupture and elasticity and no change in the work to maximum load. More brittle fractures were found when compared with fresh wood but no change in the hardness of the sprinkled wood could be detected. Loss of ray tissue and degradation of pit torus and margo was found after three years' sprinkling and in addition, some degrade to $S_3$ tracheid wall layers after four years' sprinkling. The permeability of four years sprinkled wood to a non-polar liquid was greatly increased but only slightly increased for water. The results indicate that overtreatment in commercial treatments could be experienced.

Fungi isolated from the logpile after three years could be placed in the first stages of published fungal successions in wood: however, after four years' sprinkling the appearance of some basidiomycetes indicated a change in the succession. Conditions prevailing in the logpile after four years' sprinkling revealed that oxygen was not limiting while temperatures were adequate to support fungal

growth. However, bacterial isolates did inhibit growth of a selected rot fungus. This suggests that the mechanism which restricted the growth of woodrot fungi after four years' sprinkling involved established bacteria.

Investigations of the early stage microbial succession in sprinkled *P. radiata* showed a variety of bacterial taxa. Grouping of all the isolated bacteria using degradation of cellulose, pectin and starch as the major characters was not successful.

Water sprinkler storage of *P. radiata* proved satisfactory for three years but some strength changes and rot developed after four years.

# P R E F A C E

The work reported in this thesis concerns the effect of sprinkling windthrown 45-year-old *Pinus radiata*. It was a wide ranging study covering field experiments, observation, and laboratory studies. Included in the work were aspects of microbiology, wood physiology and wood technology.

These aspects would not have been adequately covered without the general help of Dr J. Allen, the help of Drs J. Walker and J. Butcher in aspects of wood technology and mycology respectively, and that of Mr N. Clifton, Forest Service, Christchurch, who willingly supplied any samples required.

For help in experimentation thanks must be expressed to R. Dalley and K. Schashing; photographic assistance was by D. Clark; while O. Gibbons ably assisted in the hours of sample preparation.

A grant by Forest Research Institute for equipment and personal assistance and a bursary from Ivon Watkins-Dow made it possible for me to undertake this project.

Lastly I am very aware that without the support of my wife and forbearance of my children little would have been accomplished. For this I will be forever in their debt.

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# CHAPTER I

# I N T R O D U C T I O N

## 1:1 WINDTHROW 1975

Following a northeastward moving cold front on August 1st 1975, a northwesterly gale of up to 150 km/h blew down 10,700 hectares of merchantable pine forest in the Canterbury Plains (Clifton 1978). The wind, while tending to swing towards the north at times, consistently blew from a narrow arc of about forty degrees around northwest during periods of high velocity (Wilson 1976). This northwest wind is a strong, turbulent föhn wind which first appears in the gorges and riverbeds of the high country and then spreads out across the flat country (de Lisle 1969).

*Pinus radiata* D. Don aged between forty and forty-five years was the main species affected, the trees being top heavy for their generally weakly developed root systems (Clifton 1978). The predominantly shallow, stony soil, a yellow-brown earth, was a major factor in the development of a shallow root system which resulted in large numbers of windthrown trees being uprooted rather than broken. This allowed recovery gangs to concentrate initially on extracting broken timber, as the uprooted trees, although horizontal to the ground, remained alive over a period of months. Thus two recovery operations were mounted, the first for broken timber, the second for uprooted timber.

Experience in Germany from storage of windthrown timber in 1967 (Liese and Karstedt 1971; Peek and Liese 1976) indicated the possibility of storing large amounts of windthrown timber under water until the market could absorb it. In areas where large bodies of water did not exist this storage was achieved by decking and sprinkling the logs. Following the blowdown of 1975 in Canterbury an experimental sprinkler stockpile was set up in 1976 to determine the efficacy of this storage for New Zealand grown *Pinus radiata* consisting mainly of sapwood.

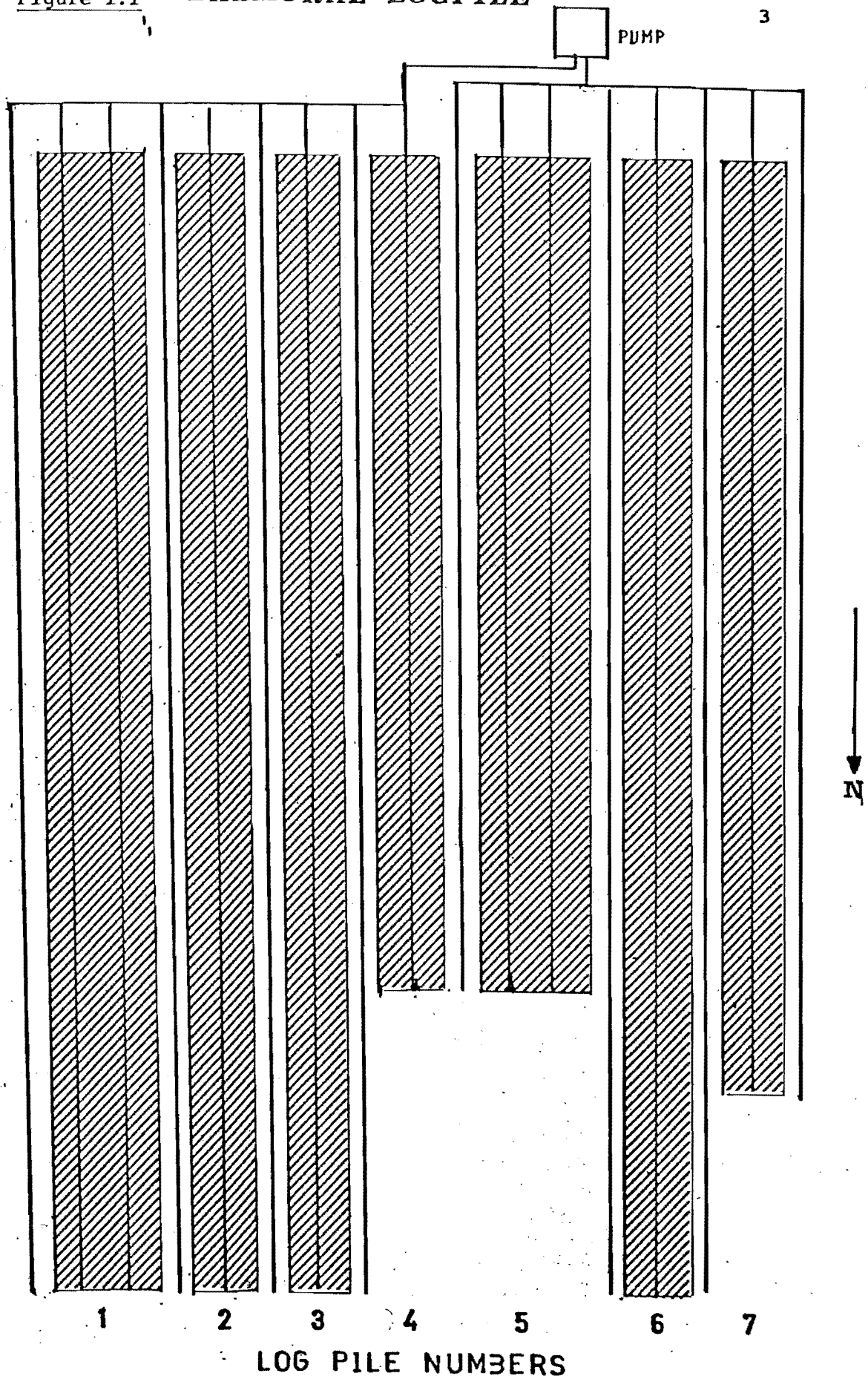
## 1:1:1 Sprinkler Stockpile

The experimental stockpile was set up on 1.6 hectares of free draining alluvial gravel, close to the Hurunui River in the Balmoral State Forest. Logs, cross cut in the forest, were stacked in rows within 48 hours of cutting; seven rows, 160m long and 4.5m high, were built (Figure 1:1). In all, 14,550$m^3$ of timber was stored in the stockpile.

Water was obtained from two 250mm diameter wells sunk to a depth of 8.8m. Spraylines were laid over each row of logs, between each row of logs and along all ends. Sprinklers which delivered 8 litres/minute over a radius of 10.5m at 280 KPa were fitted at intervals along each sprayline. The sprinkler system as a whole was divided into two sections with water being delivered to alternate sections at 20-minute intervals.

All logs received for the experimental stockpile were examined for sapstain and affected logs were rejected. The stockpile was constructed between April and November 1976 with the intended duration being five years. During this

Figure 1:1 BALMORAL LOGPILE



period a number of checks were to be made as to condition of the logs and general biological activity of the logpile (Hosking 1977). A research contract was awarded to Forestry School, University of Canterbury, to further investigate the effects of sprinkler storage on radiata pine; this was commenced in January 1979.

### 1:1:2 Summary of work to 1979

Twelve months after storage commenced a quantity of timber was removed, sawn into boards and found to be clean and free from any obvious signs of biological deterioration (Clifton, *pers. comm.*). Anaerobic bacteria were isolated in small numbers and some rodlike bacteria were located in the region of the pit membrane in a small number of bordered pits.

After two years of storage seventeen cubic metres of logs sawn in April 1978 showed no difference in general appearance from fresh Balmoral grown timber and no difference in preservative uptake by the boron diffusion process (Clifton 1978). Haslett (1980) comparing timber sawn from two year stored logs and fresh sawn Waipa grown timber found no difference in drying rate or shrinkage although the stored material showed a slightly increased tendency to surface check when dried for the second time after pressure treatment with Tanalith NCA.

### 1:1:3 Scope of Present Project

The purpose of this present study was to monitor the development of the fungal and bacterial flora in the stored logs and identify the role of the dominant organisms. This succession was to be related to changes in wood microstructure

and wood properties. The purpose was to provide background information on the effect of water storage on commercial drying, preservation and the strength properties of converted timber.

Emphasis was placed on characterising the development of the bacterial and fungal floras in stored wood. Identification of organisms of major numerical importance was made, but more emphasis was placed on physiological rather than taxonomic groupings.

## 1:2 SURVEY OF WOOD STORAGE BY PONDING

Storage of wood in water as a means of inhibiting decay has been known for a considerable length of time. In 1927 Lagerberg *et al.* discussed water as a means of storage and commented that it is old knowledge that timber lying immersed or stored in water is not appreciably damaged during storage. Furthermore they commented that where this form of storage is available it is superior to all other methods.

Wood can be stored in water for a variety of reasons. Cartwright and Findlay (1958) stored logs in ponds to inhibit the development of fungi and to prevent end splitting. Other reports also indicate that this method of storage was employed to prevent decay in timber. Osborne *et al.* (1956) stored logs in a pond for up to 14 months with no deleterious effect on pulp yield or quality. Chesley *et al.* (1956) also found that the yield and quality of kraft paper was unaffected by water storage of the timber for up to 12½ months. However seven months' storage of southern pine pulpwood in the yard

led to a loss of 3% oven dry weight and bursting strength and tear values were reduced by as much as 25%. Rawlinson (1968) suggested that green logs can be stored successfully under water for up to two years.

Rotary cut veneer from southern pine which had been stored under water for six months showed no significant reduction in flexibility, toughness or specific gravity (Lutz *et al.* 1966).

However, Unligil (1971) reported that bolts of white spruce stored in a lake for four months showed some loss of strength. He found that water stored wood was 10.4% lower in fibre stress at proportional limit, 5.4% lower in modulus of rupture, but that the modulus of elasticity was not significantly lower when compared with white spruce stored outdoors for the same length of time.

Another aspect of ponding is discussed by Ellwood and Ecklund (1959a) who showed that sapwood of ponderosa *(Pinus ponderosa)* and sugar pine *(Pinus lambertiana)* developed an extreme porosity when stored in a pond containing virtually stagnant water. They found that logs stored in the pond for one to several months rapidly increased in permeability without any substantial change in strength (Ellwood and Ecklund 1959b). This excess porosity developed because of a corrosion and eventual destruction of parenchyma particularly in ray tissue. Spore forming, facultatively anaerobic bacteria were identified as the causative organisms.

Bolton and Petty (1975) investigated this further when studying the gaseous permeability of wood by storing wood in ponds to promote bacterial decomposition of the

bordered pit apertures. A water temperature of 20°C to 30°C was found to give a greater increase in permeability than 10°C (Banks 1970).

Advantage can be taken of this increase in permeability of water stored wood. Holmgren (1961) discussed the deliberate waterlogging of logs to allow a better penetration of preservatives. He noted that short periods of storage in lakes allow a greater and more even penetration of preservatives into small diameter logs.

The organisms responsible for the development of the highly porous areas in the sapwood have been identified but the activity of the principal organisms is not confined to stagnant, highly contaminated log ponds. *Bacillus polymyxa*, for one, is active in almost any environment in which logs are stored (MacPeak 1963).

Further investigation on the permeability of ponded wood found that longitudinal, tangential and radial permeability of sapwood is increased (Dunleavy and McQuire 1970) but there was no indication of any effect on heartwood permeability. Bauch *et al.* (1970) found that ponding of *Pinus silvestris* for three weeks was all that was needed to reach the maximum level of permeability. Five weeks of pond storage were needed for full sapwood penetration of creosote into white spruce *(Picea glauca)* using an eight hour Lowry process (Unligil 1972). However in Goose Bay, Labrador, twelve weeks during July, August and September of ponding with debarked white spruce were needed to get an uptake of creosote nearly equal to the sapwood thickness: temperature could be the important factor in this situation.

## 1:3 SURVEY OF WOOD STORAGE BY SPRINKLING

Water storage of logs has been accomplished by sprays as well as ponding. Hansbrough (1953) suggested the use of continuous sprays for the storage of timber. Various reports on spraying as a method for wood storage testify to the effectiveness of this method for periods of up to twelve months (Mason *et al.* 1963; Finighan and Liversidge 1964; Volkman 1966; Skolmen 1966; Roff and Dobie 1968; Djerf and Volkman 1969). Scheffer (1969) suggested that water sprinkling acts by restricting the diffusion of oxygen into the logs, and saw the cooling effect as being of little importance. Lane and Scheffer (1960) looking at birch and maple logs deck stored under water sprays concluded that the variety of microorganisms occurring at the ends of logs producing a slimy surface may have aided in the control of rot and stain by consuming and therefore filtering out most of the oxygen. They also suggested that there could be some antagonism.

Some investigation into the effects on longleaf pine of storage under water sprays (De Groot and Scheld 1971; Scheld and De Groot 1971; DeGroot 1972; DeGroot 1973; DeGroot and Sachs 1976) elicited the following conclusions. Lumber from the stored logs was found to be no more susceptible to decay than lumber cut from logs soon after the trees were felled. Three basidiomycetes, *Peniophora gigantea*, *Lenzites saepiaria* and *L. trabea* were used to determine the susceptibility of the wood to decay. A significant loss in toughness was found after storage of four to eight months. Toughness was measured by ASTM D 143-52. It was assumed that

the loss in toughness was due to the destruction of some non-cellulosic component. Absorption, measured by submerging a 25mm cube of sapwood in mineral spirits for four minutes, increased, as did the radial air permeability of the sapwood. The increase was slight during the first month, rapid during the second month of storage and very slight thereafter. The steep increases in the absorptivity and permeability were associated with the destruction of cell walls of the ray parenchyma cells. *Bacillus spp.* were present in the sapwood of living longleaf pines. However when stored under water spray the wood was invaded by gram negative, fermentative bacteria mostly from the Klebsiella - Aerobacter - Serratia group.

Other workers have found bacteria invading water sprayed wood. Karnop (1972) isolated six species of non-sporing, gram negative motile rods from the sapwood of scots pine. He found these bacteria digested, to varying degrees, protein, starch, sugars and pectin but not cellulose or hemicellulose. However of twelve other isolates including five *Bacillus spp.* and two *Clostridium spp.*, seven could split hemicellulose as well as pectin and starch but no isolates attacked cellulose.

Fogarty (1973) isolated *Bacillus polymyxa, B. subtilis, B. mesentericus, Staphylococcus sp, Clostridium omelianski* and *Flavobacterium pectinovorum* from water sprayed wood. No attempt was made to determine the enzymatic capabilities of each isolate with respect to wood breakdown but amylase, xylanase and pectinolytic enzymes were detected in expressed sap.

Thus the storage of logs under water spray has been shown to cause few losses in wood substance or strength, the major loss reported being one of toughness. There is an increase in permeability and an invasion of bacteria which, although somewhat different for different situations and wood type, are the major modifiers to the stored wood. The modification caused by the bacteria, as mentioned is small and this has led to the use of longer periods of storage. Moltesen (1977) investigated the storage of sprinkled and ponded spruce *(Picea abies)* and beech *(Fagus sylvatica)* and recommended maximum storage times of eight months for furniture wood, two years for veneer logs and four years for pulpwood.

The storage of wood for long periods of time under water may be necessary for a variety of reasons. Development of this means of storage was originally undertaken in areas where because of seasonal variation in climate, storage is necessary for some months to ensure continuity of supply to the processing plant. As a result much information is available on storage periods up to a maximum of six months (Ellwood and Ecklund 1959; Scheld and DeGroot 1971). Recently, longer periods of storage have been embarked upon in an attempt to fully recover timber which has been windthrown. Thrown timber must be either utilised or stored within a few months, the emphasis being to get the timber off the forest floor quickly. To this end studies have been made as to the maximum possible period of storage under water. As mentioned above, Moltesen (1977) attempted to define the maximum period of storage permissible. Dalgas *et al.* (1975)

reported on a three year study of sprinkling windthrown timber. They found no change to the modulus of rupture, some reduction in the modulus of elasticity and some degrade due to soft rot. However in this study sprinkling was only continuous through the summer months and was discontinued during winter. Arnold *et al.* (1976) and Peek and Liese (1976) gave accounts of some 1.4 million cubic metres of softwood *(Picea abies* and *Pinus sylvestris)* windthrown in Germany. They concluded that if properly carried out with the moisture content of the sapwood kept at greater than one hundred percent, over two years storage is possible.

# PART I

# PHYSICAL CHANGES IN SPRINKLED WOOD

# CHAPTER II

## MICROSCOPIC CHANGES IN WOOD

In 1946 Virtanen found bacteria, capable of utilizing cellulose, fermenting wood dust. This was among the earliest reports that bacteria had any role in wood breakdown. Courtois (1966), however, stated that the effect of bacterial decomposition was insignificant when compared to fungal breakdown of wood. This comment was made despite his observing bacteria in bordered pits, and depressions in the cell wall. Greaves (1971) also noted that by comparison with wood destroying fungi, the decay rate produced by bacteria is slow.

High moisture content in wood however appears to favour bacterial rather than fungal breakdown. Knuth (1964) in a study of the effects of bacteria on the chemical and physical properties of wood products showed that *Bacillus polymyxa* was present in most wood he looked at, but that only parenchyma cells were attacked.

Boutelje and Kiessling (1964) observed bacteria in the parenchyma cells of oak which had been in sea water since the seventeenth century. Some damage to parenchyma walls was noted although it was not possible to conclude that this was caused by the bacteria. Harmsen and Nissen (1965) investigating 90-year-old foundation piles of *Pinus silvestris* and *Picea abies* found evidence of cellulose decomposing bacteria. In rays and tracheids small conical depressions extending from cell lumen into the secondary wall were found.

These depressions could be found in the sides of the secondary wall but did not penetrate the middle lamella. The sequence of attack was: tracheids adjacent to the rays were attacked first; next, tracheids at the growth ring boundary; then the attacks became more scattered.

Rod shaped bacteria were found in ray cells and tracheid lumena by Boutelje and Bravery (1968). However in the bordered pit cavities cocci were found. Four types of cell wall attack were noted:

(1) Attack originating from within the lumen as degraded areas through the $S_3$ into the $S_2$ layer.

(2) Spiral bands showing widespread disorganisation of the $S_2$ layer, often coincident with borded pits.

(3) Non-spiral bands of widespread disorganisation within the $S_2$ layer.

(4) Well defined, irregular, angular areas showing loss of birefringence.

Greaves and Levy (1968) and Greaves (1969) have described various kinds of bacterial attack. In infected material of *Pinus radiata* all structures of the wood's anatomy may be colonized by bacteria (Greaves 1969). Pit borders show three main types of attack:

(1) The border degraded in discrete places.

(2) Perforations occuring at the very edge of the border.

(3) Decay of the wall layers from the region of the annulus. Pit margo and torus may be decayed, incrustations which normally occur on the margo and torus may be selectively removed. Tracheids are also subjected to

thinning and removal of the secondary wall, the endpoint being when the middle lamella is reached. However, Greaves (1969) emphasises that rays constitute the main source of carbohydrates for invading microorganisms, and are normally first colonized. Ray cell breakdown frequently follows the phase of colonization and development and Greaves recognises two types, i) a non-destructive attack in which the birefringence characteristics of the ray parenchyma cells are affected and ii) a destructive attack of the parenchyma cells in which the entire walls are broken down. This separation however may be more apparent than real because cellulose fibril organisation must be changed to cause a change in birefringence.

Boutelje and Göransson (1971) while recognising these patterns of attack also noted the presence of non-cellulytic bacteria which had penetrated to a considerable depth. Greaves and Foster (1970) however present a series of photomicrographs of cells in various stages of breakdown with a wide range of morphologically different bacteria associated with these cells. They suggest that all the various types found, contribute to the degradation of the cell surfaces citing lysis holes, erosion troughs or "halos" of removed material which surround each bacterial type.

Greaves (1973) found that a selection of twenty wood inhabiting bacteria could all produce small but highly significant weight losses in laboratory tests. He found that the mechanism of bacterial attack, apart from gross features such as wall pitting, was at the ultrastructural levels. Enzymes break down regular microfibril or micell

structure by attacking cellulose chains at cross linkages. *Bacillus polymyxa* has been shown to attack softwood walls (Greaves 1965, 1970; Greaves and Levy 1965). Schmidt (1978) also found *B. polymyxa* capable of attacking the cell wall of *Pinus sylvestris* but only after chemical pretreatment reduced the lignin slightly. In fact, of thirteen strains of bacteria all known to produce cellulase, only two, a *Cellulomonas* and *B. polymyxa* affected the cell wall. Schmidt goes on to suggest that the chemical pretreatment may modify either the lignin incrustation of the carbohydrates or the lignin molecule as such; in either case the cellulose substrate may become accessible to bacterial enzymes. These findings support work by Bailey, Liese and Rösch (1968) who found that the inability of many cellulolytic organisms to attack lignified cells was because they did not have the ability to effect a pre-cellulolytic stage.

## 2:1 BACTERIAL INDUCED CHANGES IN WATER SOAKED WOOD

Ellwood and Ecklund in 1959 reported that after ponding *Pinus ponderosa* and *P. lambertiana* logs for several months bacterial attack could be found. This was characterised by removal of the contents of parenchyma ray cells and finally by destruction of ray tissue. Many reports since this time have confirmed these findings. MacPeak (1963) found the development of highly porous areas in ponded wood; Volkman (1966) the loss of ray and resin canals; Karnop (1972) attack of pit membranes of tracheids and parenchyma; Greaves and Barnacle (1970) found clearing of ray cell contents, attack of bordered pits and removal of

tracheid walls in water sprayed pine logs. Bauch, Liese and Berndt (1970) found that the ponding time for *Pinus sylvestris* necessary to show somechange could be as short as three weeks. Liese (1970) however suggests that degradation by bacteria is a prolonged process for water stored logs. He found that invasion by bacteria was mostly through the ray parenchyma, and that the signs of attack were variable; most frequently however the tori of bordered pits became granular and disappeared leading to deterioration of pit membranes.

## 2:2 LIGHT MICROSCOPE STUDIES

Wood samples were taken from the logpile after three and four years sprinkling (Appendix A2). These were sectioned using a sledge microtome and stained with safrinin and light green using the schedule detailed in appendix B1. Suitable 25nm thick sections of transverse, radial-longitudinal and tangential-longitudinal orientation were mounted on glass slides. The mounted sections were examined by bright field, interference phase and polarized light microscopy and compared with sections of fresh wood taken from similar aged trees in Balmoral Forest.

After three years of sprinkling there were signs of cellular breakdown. Much of the ray tissue had disintegrated, no cellular contents were found and some cell walls had collapsed, (Fig. 2:1). Most of the bordered pits in tracheids had lost their tori; the differential staining showing no sign of pectinaceous material (Fig. 2:2, 2:3).

In the sample after three years' sprinkling only sapwood was examined.

Four years of sprinkling resulted in complete loss of any cellular detail in the sapwood rays with the total absence of dividing parenchyma cell walls. The parenchyma cells surrounding resin canals had also lost most of their cell walls and no tori remained. In the heartwood very few bordered pits had an intact torus, however some ray tissue remained while parenchyma cells could be discerned. These findings correlate well with those studies already mentioned. Greaves and Barnacle (1970) found a similar loss of the structures mentioned above.

Using interference and polarized light microscopy crystalline material can be recognised and the crystal axes can be determined. The birefringence is the refractive index difference (Preston 1974) and this is determined by measuring the path difference using suitable compensators. This method was used to show that some change had occurred in the birefringence pattern in the tracheid cell walls of the sprinkled sapwood. No change in birefringence could be found in the heartwood.

Greaves (1969) mentions this change in birefringence characteristics in ray parenchyma cells which have been colonized by bacteria. The secondary wall appears normal under bright field illumination and Greaves contends that the changes are at the submicroscopic level with changes to the cellulose crystalline arrangement. Boutelje and Bravery (1968) also describe this type of attack. Holt and Jones (1978) found changes in birefringence in cell walls of

bacterial attacked wood, describing chains of cavities in the middle lamella/primary wall region.

The areas where changes in birefringence had occurred in the wood of this study were scattered, showing no linking together to form a chain. The areas of changed birefringence were small and many appeared superficial, suggesting the changes were minor.

No loss of the $S_3$ layer could be detected using any of the mentioned microscopic techniques although the sprinkled sapwood did appear to have less secondary thickening than the fresh wood. However as the samples were not matched or paired wood samples a closer examination of the secondary wall layers was left for the scanning electron microscope.

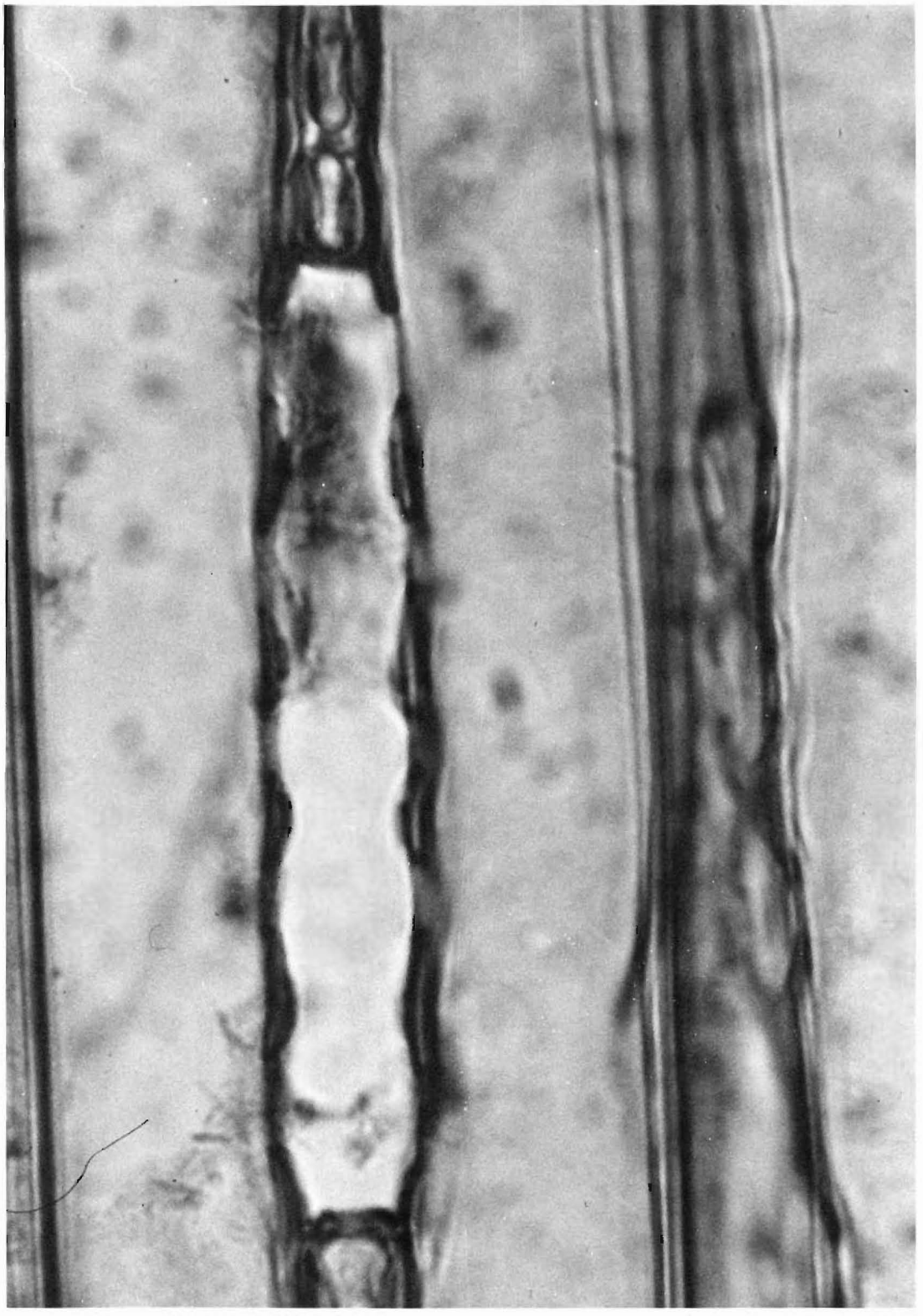


Figure 2:1

Ray tissue of *Pinus radiata* after three years' sprinkling: note loss of cell walls and lack of cellular contents. x 200

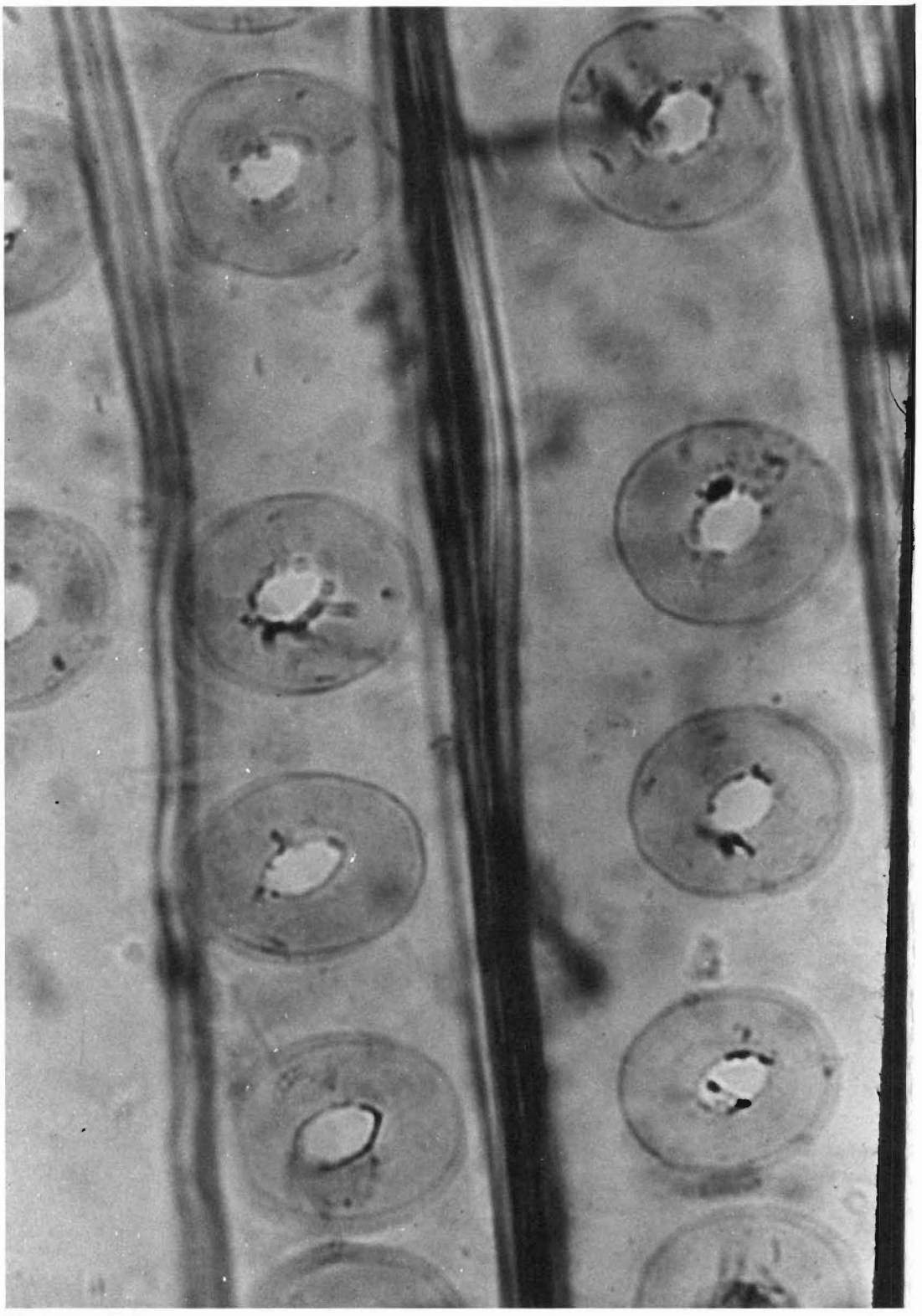


Figure 2:2

Differential staining of fresh untreated *Pinus radiata* showing staining of pectinaceous areas. x 200

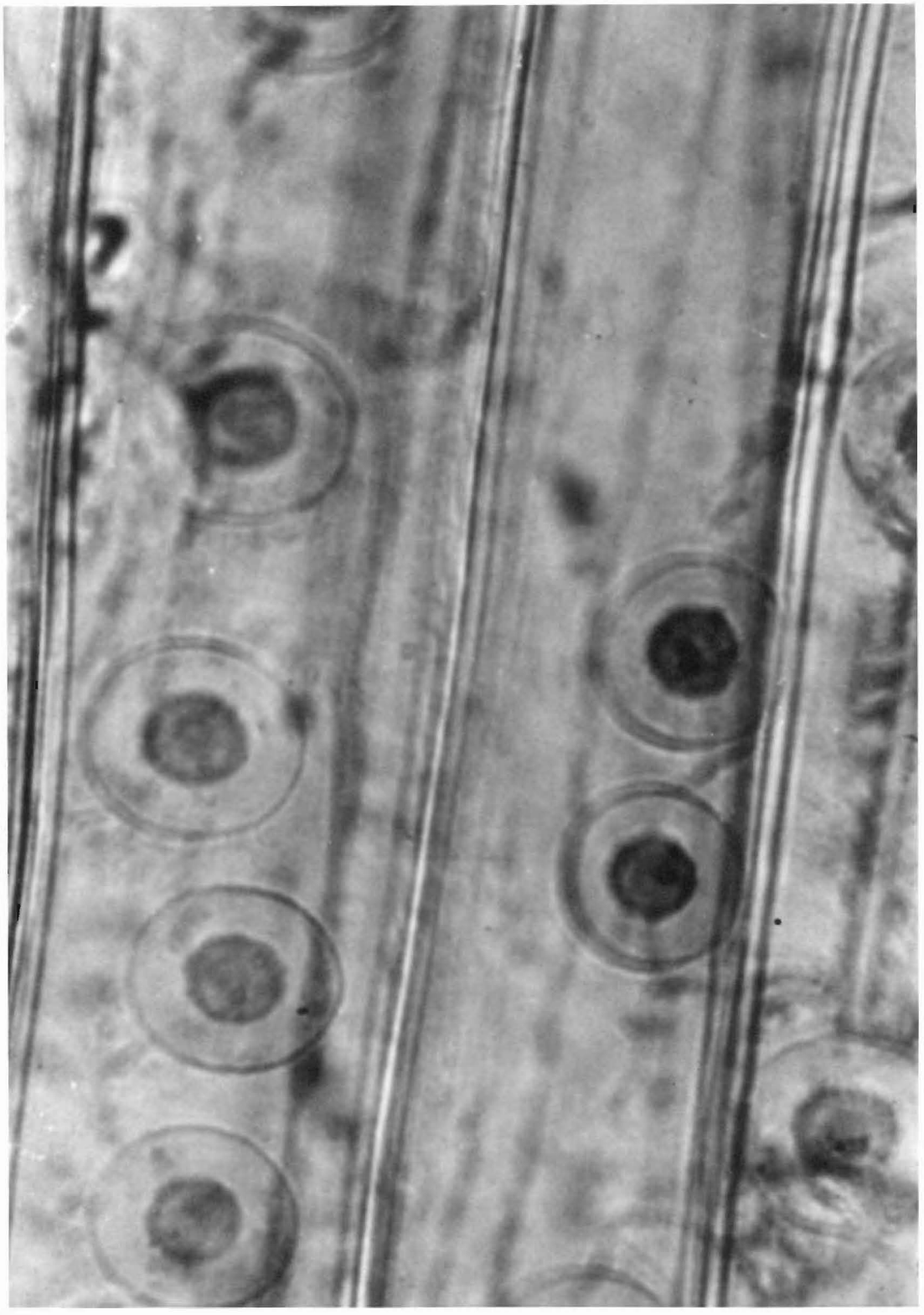


Figure 2:3

Differential staining of *Pinus radiata* after three years' sprinkling showing loss of tori. x 200

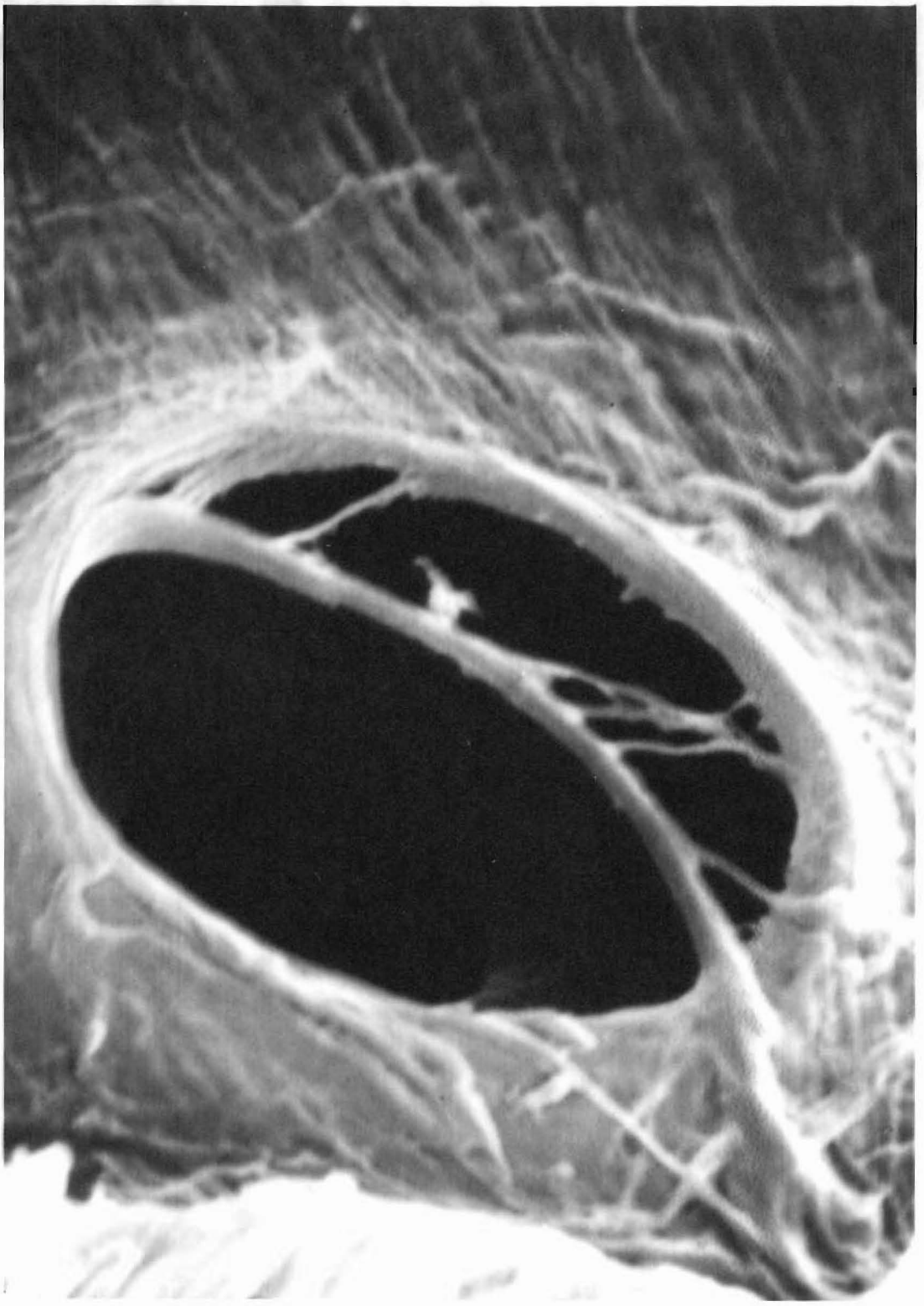


Figure 2:4

Scanning Electron Micrograph showing the loss of torus and margo after three years' sprinkling. x 10 000

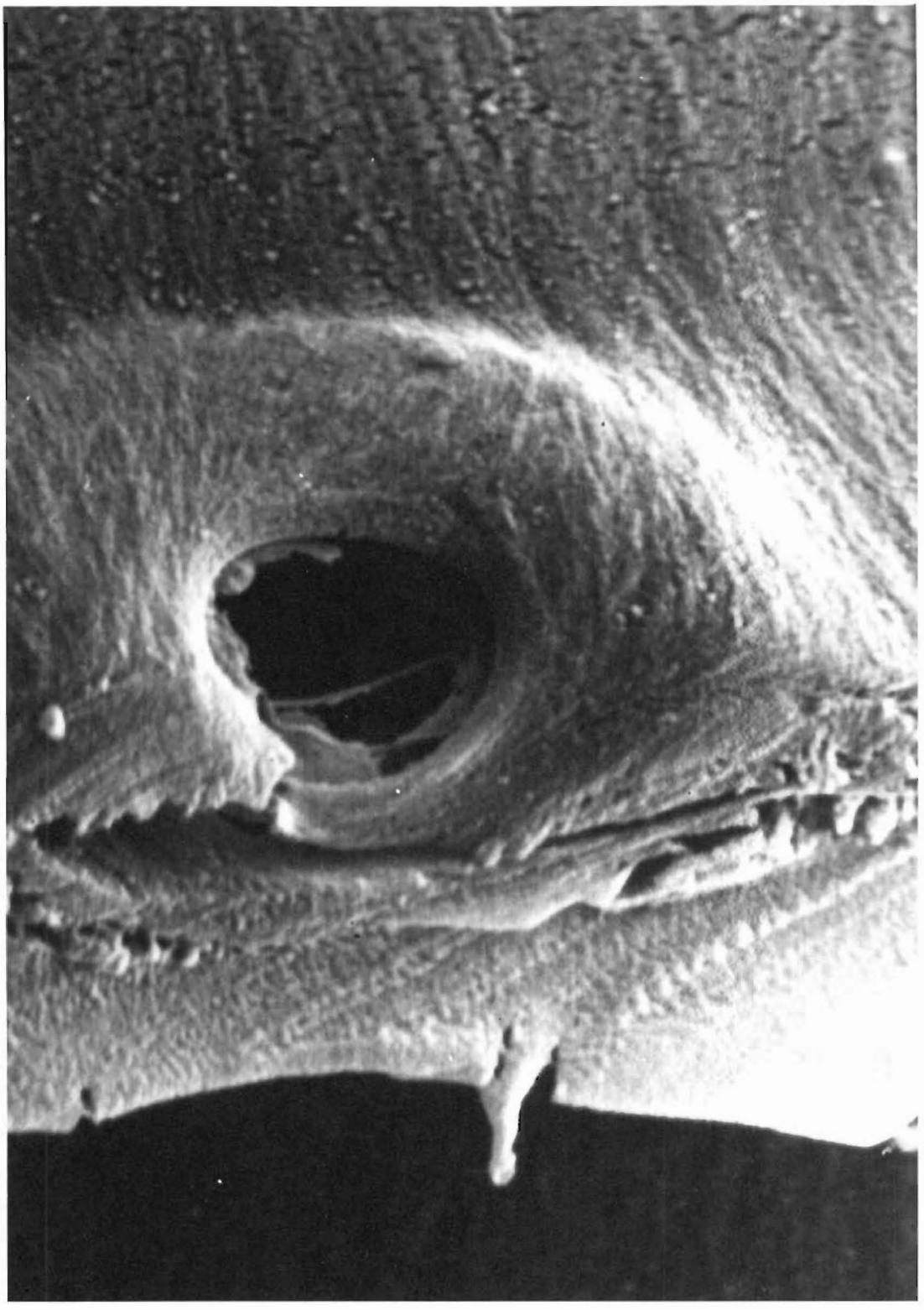


Figure 2:5

Scanning Electron Micrograph showing partial loss of margo after three years' sprinkling. x 5000

Figure 2:6

Scanning Electron Micrograph showing damage to pits between ray and trachied tissue after three years' sprinkling. x 5000

Figure 2:7

Scanning Electron Micrograph showing bacteria associated with degraded bordered pit. x 5000

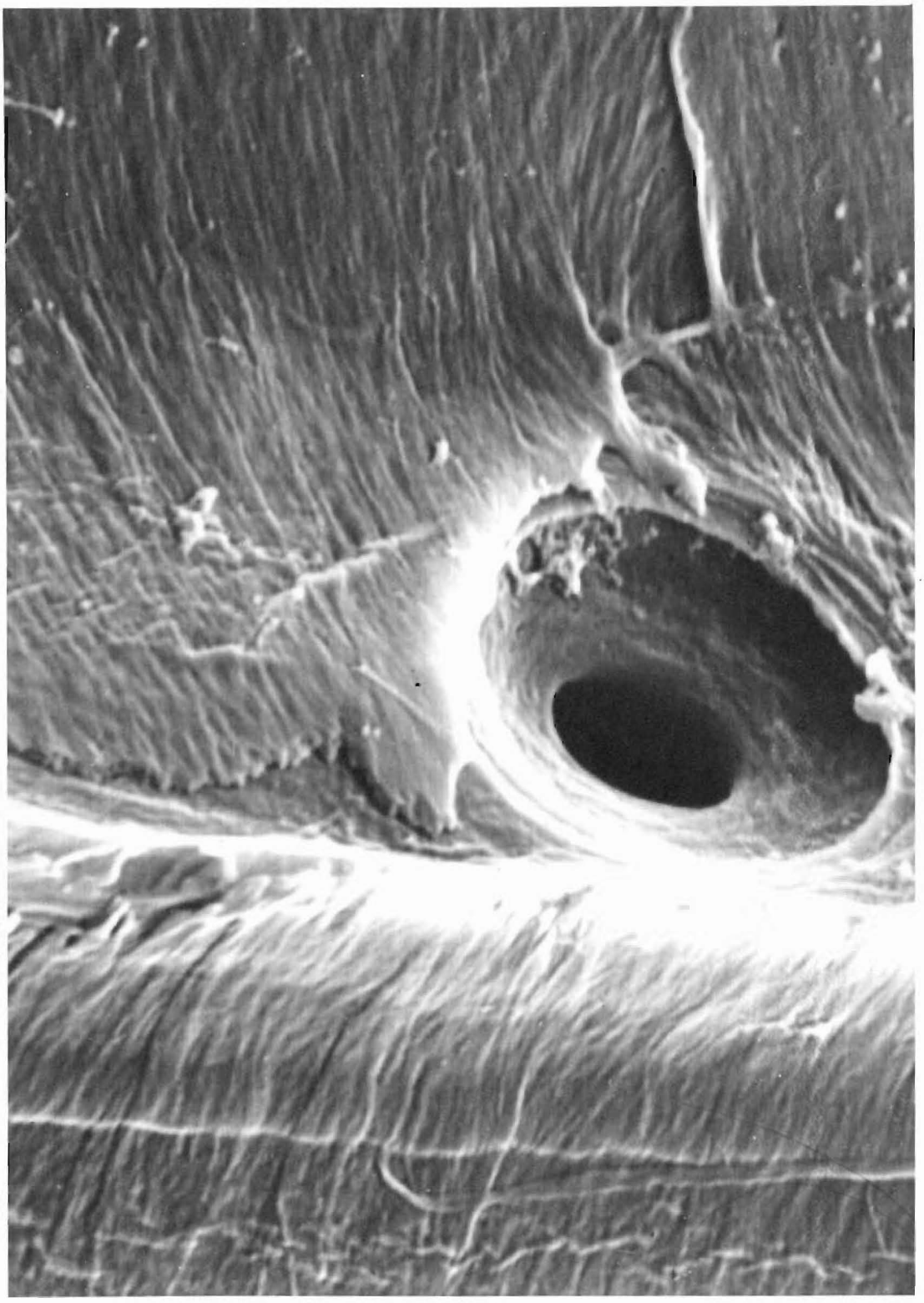


Figure 2:8

Scanning Electron Micrograph showing damage to $S_3$ layer after three years' sprinkling. x 5000

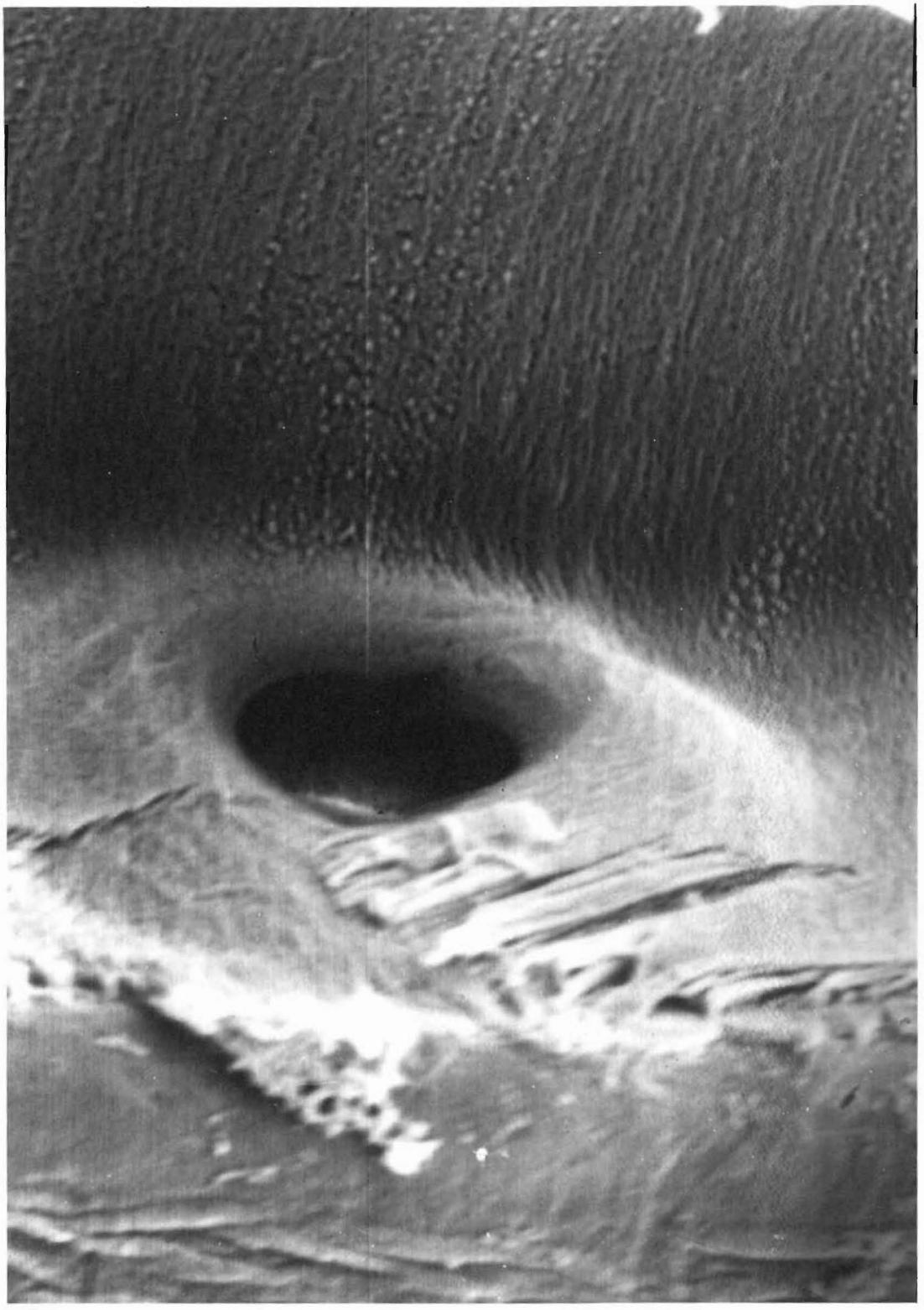


Figure 2:9

Scanning Electron Micrograph showing pitting of cell wall after four years' sprinkling. x 5000

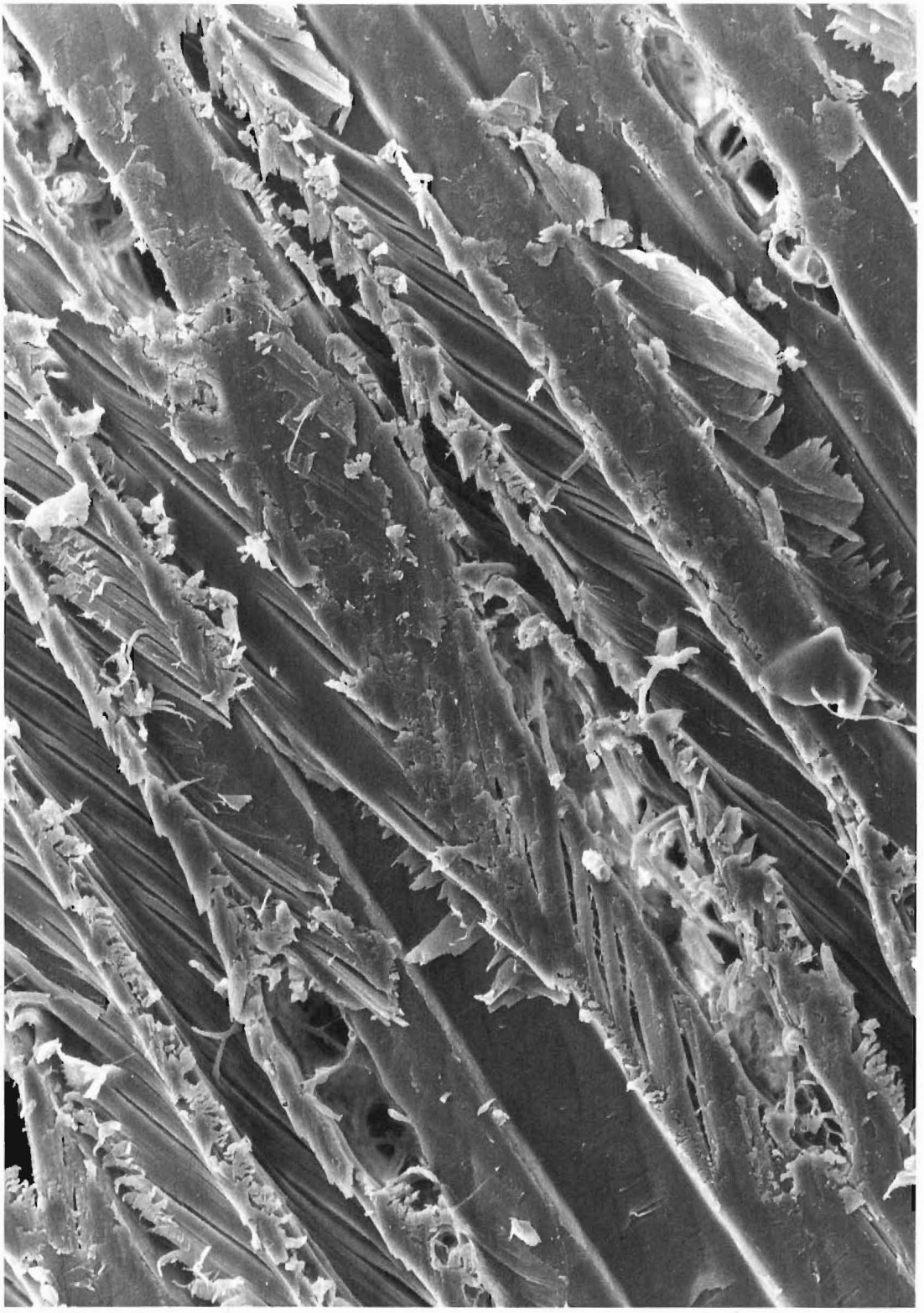


Figure 2:10

Scanning Electron Micrograph showing the regular cracking of the cell wall running in the same orientation as the fibrils of the $S_2$ layer. x 200

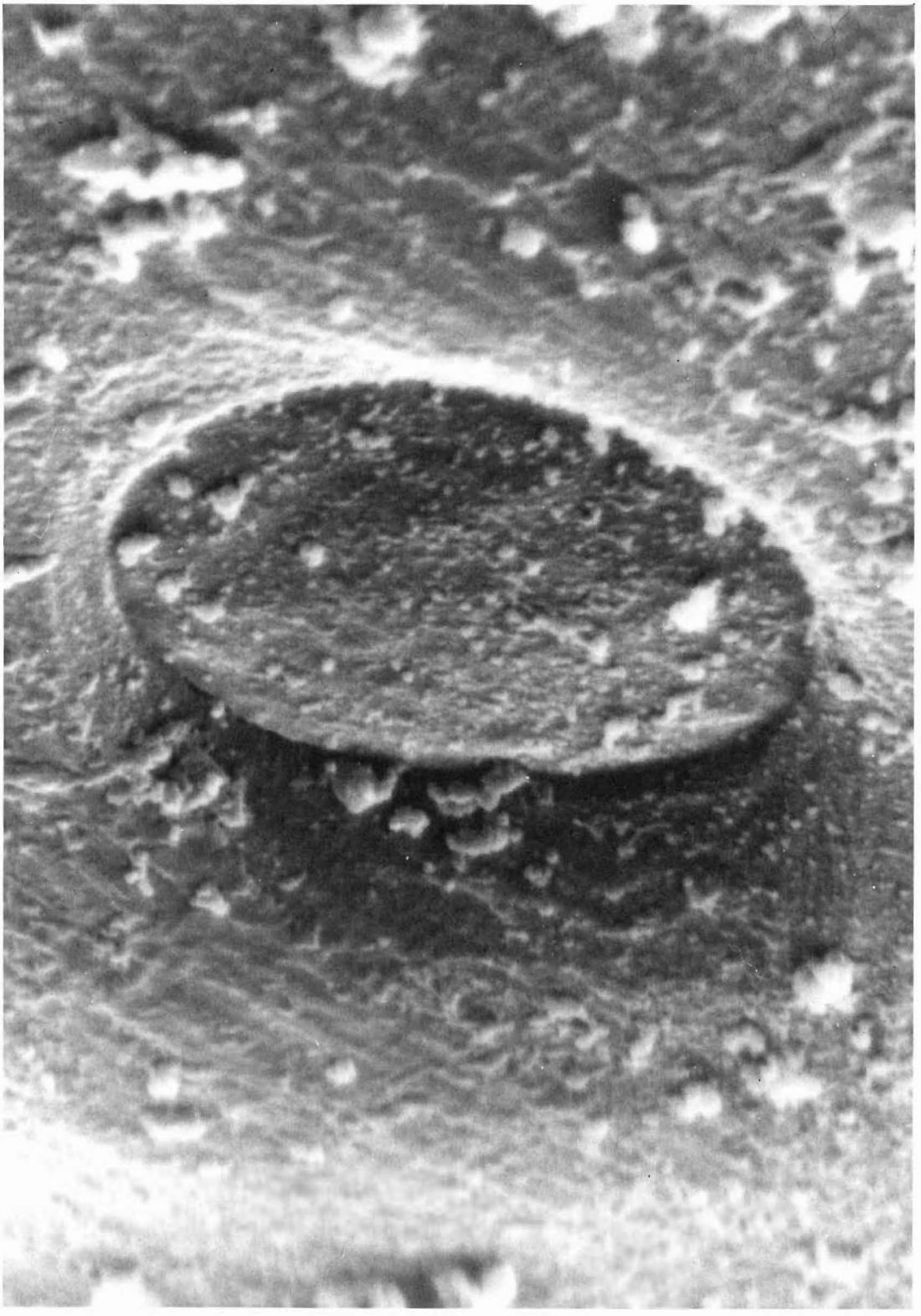


Figure 2:11

Scanning Electron Micrograph showing an aspirated pit of fresh *Pinus radiata*. x 7800

## 2:3 SCANNING ELECTRON MICROSCOPE STUDIES

As discussed in section 2:1 sections were also taken for scanning electron microscope (SEM) study, their preparation being described in appendix B2.

Most bordered pits from the three year sprinkled sapwood examined showed a loss of both torus and margo (Figure 2:4) although some pits showed a torn but not completely lost margo (Figure 2:5). Pits between ray and tracheid tissue also showed damage although in many cases the membrane was partially intact (Figure 2:6). Bacteria were found associated with many pits (Figure 2:7) as well as along the tracheid wall. Some damage was found to the $S_3$ Layer around bordered pits (Figure 2:8) but the damage did not extend far, being mainly around the edge of the pits. This loss of the border was frequently noted. SEM sections were prepared by razor blade but the edges of the border were suggestive of enzymic degradation rather than tearing or cutting, although it was puzzling that only one border of the pit was lost.

A different pattern emerged after four years' sprinkling. Damage to the secondary wall was found in the form of partially disintegrated tracheid walls. Two types of damage were found; an irregular pitting in the cell wall (Figure 2:9) which was also found in three year wood (Figure 2:5) and a regular cracking (Figure 2:10). The cracks ran longitudinally and appeared to have the same orientation as the $S_2$ cellulose fibrils. The winding angle measured appears to vary from $15^{o}$ to $20^{o}$ which suggests exposed $S_2$ fibrils. However this is not conclusive as the usual winding angle is about $14^{o}$. Compression wood has a winding angle closer to $28^{o}$. The

angle of cracking indicates fibril orientation intermediate between the two. This could be due to enzymatic damage to the cellulose fibres or their wall matrix. However the possibility of this being compression wood cannot be ignored.

The damage found using the SEM is similar to that found with the light microscope. After three years of sprinkling the sapwood showed partially damaged or disintegrated parenchyma ray cells and many damaged bordered pits. One year later signs of secondary wall damage could be found. Such damage implies that the sprinkled wood should be more permeable as was found by Adolf and Liese (1972) in spruce wood after three years' water storage.

# CHAPTER III

## PENETRABILITY OF SPRINKLED WOOD

The absorptivity of pine sapwood blocks increases in unsterile water but not in sterile water, the increase being due to bacterial activity (Ellwood and Ecklund 1959). Ponding of wood has been shown to increase the permeability and treatability of Scots pine, beech and birch sapwood (Greaves and Levy 1965) *Pinus radiata* and *Eucalyptus regnans* (Greaves 1970); Scots pine (Banks 1970); Scots pine (Banks and Dearling 1973); Scots pine (Bauch, Liese and Berndt 1970); Sitka spruce (Unligil 1971, 1972); Ponderosa pine (Bolton and Petty 1975); Sitka spruce (Dunleavy and McQuire 1970). Moreover Banks and Dearling (1973) found that storage in water with a large bacterial population even under low oxygen conditions resulted in an increase in gas flow permeability for sapwood. Banks (1970) noted that increases in permeability of 40 to 50 times could be achieved with wet storage of wood. Banks and Dearling (1973) using 2.5cm cubes found that the maximum permeability was reached within three weeks storage in aerobic conditions. This was also found by Bauch, Liese and Berndt (1970). Unligil and Krzyzewski (1974) found that with *Picea glauca* the best pressure impregnation with creosote was achieved with poles ponded without bark. Dunleavy and McQuire (1970) found similar increases with sapwood of water-stored *Picea sitchensis* but concluded that ponding had no effect on heartwood permeability.

Water storage by use of sprinklers also results in increases in the permeability of sapwood. DeGroot (1972, 1973) found the absorptivity and radial air permeability of sapwood of longleaf pine *(Pinus palustris)* increased after two months' storage. Further work by DeGroot and Scheld (1973) showed that during the first month of sprinkling there was little change in permeability followed by an abrupt increase during the next three months. *Picea abies*, after sprinkler storage however, showed poor impregnation with C.C.A. (Moltesen 1977). Ellwood and Ecklund (1959) had earlier indicated that this may also be true for ponderosa and sugar pine.

Different terms have been used to describe the uptake of a fluid by wood. The permeability of wood is a measure of the ease with which a fluid flows through a porous material under the influence of a pressure gradient (Siau 1971). This term is also used when gas is the permeating medium. The absorptivity of wood relates to the uptake of a fluid during immersion: the uptake can take place into the free space of the cells or into the cell wall structure. Penetration of a fluid is the depth to which the liquid enters the wood, while the retention is a determination of the quantity of solution absorbed by wood, usually expressed as the weight of preservative per unit volume of wood (Kollman and Cote 1968).

Various techniques have been used to demonstrate the increase in permeability in sapwood after wet storage. Banks (1970) and Johnson (1979) used nitrogen gas to determine the permeability of wood. As stated earlier Banks (1970) obtained a 40- to 50-fold increase in permeability with wet

storage of *Pinus sylvestris* while Johnson (1979) obtained increases in longitudinal permeability in western conifers but little change in radial permeability. He encountered much variability with treatments and this led him to use paraffin oil uptake as a measure of permeability in his last experiment.

The measurement of permeability has been used to determine the treatability of wood. As stated , permeability is a measure of the ease of flow of a permeating fluid, determined by measuring the rate of flow of a fluid through wood under the influence of a pressure gradient. An increase in permeability has been taken to imply that an increase in preservative loading in the wood can also be achieved (Banks 1970).

Where water is the fluid used Booker (1977) has qualified previous studies, claiming that wood permeability can be a meaningful concept only if during an experiment the rate of flow of water through wood can be maintained unchanged over a period of time. To achieve this the water and wood must be thoroughly degassed to prevent air embolism (Kelso, Gertjejansen and Hossfeld 1963). This steady state flow is not achieved during commercial treating of wood using water since the requirements are (a) a flow rate constant with time and (b) the flow be laminar (Bailey 1965).

However perhaps the best measure of the permeability of a particular wood, especially with reference to commercial preservation is the uptake of liquid using a known commercial treatment method. Bauch, Liese and Berndt (1970) and Dunleavy and McQuire (1970) using laboratory scale equipment approximated

this situation. Holmgren (1961) using a full cell treatment with creosote at 90°C or Boliden Salt Solution demonstrated an increased uptake for water stored pine. Unligil (1971) demonstrated a 179 percent increase when water stored white spruce *(Picea glauca)* was pressure treated with creosote and a 50 percent increase when pressure treated with CCA compared to controls stored dry indoors. The white spruce was ponded for four months.

Therefore to demonstrate the increase in penetrability obtained by wet storing wood and its application to the preservative treatment of wood, weight increase after immersion in a liquid has been used.

Bauch, Liese and Berndt (1970) used a vacuum pressure system to measure the uptake of aniline blue solution in ponded Sitka spruce and Scots pine. Dunleavy and McQuire (1970) also used a vacuum pressure system with Sitka and Norway spruce. Greaves (1966, 1970) used a simple system of dipping cubes of wood into aqueous kyton crimson or nigrosin solution for 60 or 20 seconds as a measure of permeability. Suolatiti and Wallen (1958) simply used water dips to determine the penetrability of water stored pine. Non-swelling liquids such as n-Hexane (Petty and Preston 1969) and Toluene (Stamm 1973) have also been used; their advantage appears to be a faster uptake compared with water.

## 3:1 UPTAKE OF NON-SWELLING LIQUID

Nicholas and Siau (1973) mention three studies where a good correlation was obtained between wood permeability and treatability. They list the most important features of

wood which influence permeability as (1) wood structure, (2) pore size, (3) pit aspiration and (4) moisture content.

Work detailed earlier (Chapter II) has shown that most of the pits in the sapwood have been degraded leaving the pits open to fluid movement. A series of experiments was therefore set up to examine the treatability of the sprinkled wood from the Balmoral logpile. A known moisture content was established by drying all wood in a kiln using a slow schedule, followed by six months' conditioning at 20°C and 60 percent relative humidity.

A simple dip test as used by Greaves (1970) was selected to test the uptake. Two trials were set up, the first to establish whether position in the logpile altered the uptake of a fluid, the other trial was to establish whether any difference could be detected between sprinkled and unsprinkled wood. The unsprinkled wood was selected from one of the stands from which wood was taken to make up the Balmoral logpile. Procedures as outlined in Appendix C2 were carried out to measure the n-Hexane uptake. The non-polar solvent, n-Hexane, was chosen as it is known that this solvent does not interact with wood, e.g., by extraction, swelling, hydrolysis, etc. (Petty and Preston 1969; Bolton and Petty 1975).

A random sampling using a nested analysis design, as outlined in Appendix C1 was followed. The results showed no significant difference in the uptake of the wood from different areas within the logpile (Appendix C3). The wood at the top of the pile did not take up significantly more n-Hexane than the wood from the bottom of the pile and

inner sapwood took up as much n-hexane as the outer sap. Therefore after three years' sprinkling it would appear that within the bounds of experimental error the penetrability of wood to n-Hexane is the same throughout the logpile, most of the variability occuring within samples, treatments adding little to the total variance.

The next point to establish was whether this uptake of n-hexane differed significantly to uptake of freshly sampled wood. Wood was sampled randomly from piles one and three (Appendix A1) and from fresh wood. The fresh wood was taken from six logs of trees located in a compartment at Balmoral from which the windthrown logs were taken to make up part of the logpile. All wood was collected at the same time and treated as in Appendix C2. One hundred samples from sprinkled wood and fifty samples from fresh wood were taken. The results as summarized in Table 3:1 show that there is a significant difference between the n-hexane uptake of fresh and sprinkled wood.

Table 3:1 n-Hexane uptake of fresh and sprinkled wood

| | Maximum ($l/m^3$) | Minimum ($l/m^3$) | Mean ($l/m^3$) | Std Dev. | Variance ratio F |
|---|---|---|---|---|---|
| Sprinkled | 292.8 | 2.9 | 49.97 | 71.11 | 538.6 ** |
| Fresh | 15.2 | 2.4 | 7.54 | 3.06 | |

The difference found could be expected following the microscopic examination which showed loss of bordered pits and ray tissue. The loss of ray tissue would influence the radial uptake of n-hexane and the loss of pits would

influence the longitudinal and tangential uptake. Testing the null hypothesis that the sample variances are similar gave a difference at the 0.01 level. However of more interest is the very large variation in uptake within the sprinkled wood samples. The assumption implicit in the analysis was that the sample was homogeneous. The earlier sampling had indicated that the differences within the logpile were not large, (Appendix C3) most of the variation being accounted for within the samples. This pattern is repeated in this second sampling in the sprinkled wood but not the fresh wood.

Regression analysis between the n-Hexane absorption and either the wood density or the number of rings per 2 cm are tabulated in Appendix C4. The analysis indicates that the slope of density and n-hexane are dissimilar but that the slope for the number of rings and the n-hexane uptake are similar. Earlier work has shown that using petroleum-base preservatives (Nicholas and Siau 1973), sapwood is easier to treat than heartwood. Petroleum-base preservatives cannot break hydrogen bonds (Nicholas and Siau 1973) therefore aspirated pits can prove an obstacle to the fluid. The significant difference between sprinkled wood and fresh, with much lower uptakes recorded for fresh (Table 3:1) found in this experiment tend to support this theory, particularly as work outlined in Chapter II showed a loss of pits in sprinkled wood. This means that solvent uptake should be greater in sprinkled wood; as was found to be the case.

However since much of the treatment done in New Zealand is with water borne preservatives the effects of sprinkling on water uptake would be more relevant to the needs of industry.

## 3:2 UPTAKE OF WATER

An assessment of water uptake was made using wood cut from the same sampling of the logpile and fresh wood as that for n-hexane uptake. Samples 100 x 20 x 20 cm were conditioned as in section 3:1, the ends were sealed with epoxy resin and treated in a small scale pressure vessel. The samples were numbered, weighed and added to the cylinder: a vacuum of 175 KPa was pulled for 15 minutes, the cylinder was flooded, vacuum released and the samples left soaking immersed for two hours. After removal and drainage for 15 minutes, a wet weight was obtained and uptake of water in $kg/m^3$ was calculated. The fluid remaining was also measured and a total uptake calculated.

Table 3:2

Uptake of Water by Sprinkled and Fresh Balmoral Wood

| | mean uptake $kg/m^3$ | Standard Deviation | Variance Ratio F |
|---|---|---|---|
| Sprinkled | 443.6 | 194.9 | 1.23 ns |
| Fresh | 327.7 | 216.2 | |
| Total | 464.0 | | |

(calculated from loss fluid in vessel)

The uptake calculated from the loss of original volume of fluid from the cylinder gave an overestimate although no correction was made for the fluid not measured while the wood was drying. The figure obtained was well within the deviations. The variance ratio F between the two treatments sprinkled and fresh, showed no significant difference, although the complete analysis (Appendix C5) does show a

significant difference between the two. However, the difference found between sprinkled and unsprinkled wood was not as large as that found with n-Hexane treatment. The implication is that when treating with a water borne preservative the lack of pits in sprinkled wood is not as important a factor in determining the amount of preservative uptake as when a petroleum based preservative is used. In a trial with white spruce, Unligil (1971) found a similar occurrence; that the differences in uptake between water stored and air stored wood (i.e. dried after cut and stored) were not as great for CCA treatment (water borne) as creosote treatment. Using a similar treatment method, (90 percent vacuum and then pressure) Holmgren (1961) did not find any significant difference between water and land stored wood treated with creosote or Boliden salt solution.

Regression analysis showed similar slopes between density and the uptake of water. The correlation between change from heartwood to sapwood, measured by the density was positive for increasing uptake, i.e. heartwood took up less than sapwood.

Analysis was also carried out to compare n-Hexane uptake and water uptake. In this case these did show significantly different regression slopes. The uptake of n-Hexane could not be correlated to the uptake of water. This is not surprising since, as stated earlier, water and petroleum liquids behave differently within the wood (Nicholas and Siau 1973).

Of more importance is the lack of difference in the uptake of water found between water stored and fresh wood.

There is the possibility that the soaking time of two hours was sufficient to break some of the hydrogen bonds, thus de-aspirating some pits (Nicholas and Siau 1973). However Preston (1974) contends that water can allow bonding of the pit membrane to the border while organic liquids cannot. He goes on to quote Petty (1970) as showing that a surface tension of 0.12 dyne $mm^{-1}$ is all that is required to move the membrane to the border while most liquids have a surface tension greater than 1.5 dyne $mm^{-1}$. However air drying does lead to aspiration (Preston 1974) and most wood is treated after drying as was the wood in these trials. The lack of difference between sprinkled and fresh wood's uptake of water might still be explained by de-aspiration. Bailey and Preston (1970) have shown that the annulus and not the pores in the margo constitute the major resistance to flow. Therefore if fresh wood's pits were largely unaspirated then the presence (as in fresh) or absence (as in sprinkled) of margo and torus is not the major consideration when considering flow resistance; hence the similar results with fresh and sprinkled wood.

Preston (1974) has also put forward the argument for capillaries in cell walls which contribute to the flow of liquids through wood. Some breakdown or disorganisation in cell wall tissue as seen by S.E.M. (Chapter II) should increase this movement in sprinkled wood when water treated but not when treated with a non polar liquid (which does not penetrate the wall). This difference is seen between the n-hexane treated and water treated wood. However this does

not help in explaining the small difference in water uptake between sprinkled and fresh wood.

Increased uptake of liquid by wood suggests a loss of cell contents. The increased uptake has been found in sprinkler stored wood and microscopy has demonstrated some degradation. These differences appear to be important for treatment of wood with petroleum base preservatives although not so much for treatment with water base preservatives. The next point to be considered is whether these changes affect the gross structural integrity of the wood. To determine this, strength tests were conducted on the wood.

# CHAPTER IV

## STRENGTH CHARACTERISTICS

Loss of strength of softwood degraded by bacterial action has not always been found (Ellwood and Ecklund 1959; Lutz, Duncan and Scheffer 1966), but some workers, (Scheld and deGroot 1971; Unligil 1971, 1972; Dalgas, Moltesen and Koch 1975) have shown significant losses to measured strength characteristics.

Different tests have been used to assess the effect of bacterial degradation of wood, the tests often chosen with respect to the end use of the timber. Scheld and deGroot (1971) tested the toughness of southern pine *(Pinus palustris)* logs after sprinkling and found a 12 percent loss after four to eight months. Nine weeks' water storage of white spruce *(Picea abies)* resulted in a reduced modulus of elasticity (Unligil 1971). However Beech *(Fagus sylvestris)* sprinkled for three years showed a change in modulus of elasticity and no change in modulus of rupture (Dalgas, Moltesen and Koch 1975). Moltesen (1977) found a loss in bending strength of three percent and modulus of elasticity of 10 percent in sprinkler stored beech. From these and other tests Moltesen was able to give a guide to the maximum average time beech should be stored under sprinklers; furniture wood eight months, veneer logs 1.5 to two years, parquet flooring two to three years and pulpwood four years.

Other researchers have also suggested maximum storage times; the times often reflecting the end use of the wood. Hatton, Keays and Waelti (1976) found that floating wood when salvaged from a hydroelectric reservoir, gave pulp yields similar to fresh wood, even after three years of water soaking. However even here there is a time limit. Boutelje and Bravery (1968) found a loss of bending strength and modulus of elasticity in scots pine and norway spruce piles which had been below the water table for 75 years and subject to bacterial degradation.

A detailed study by Bauch, Liese and Berndt (1970) showed the relationship between time of ponding, uptake of liquid and impact strength for *Pinus silvestris* and *Picea abies*. They found that after three weeks' ponding impact strength decreased to 80 percent of controls, while liquid uptake increased markedly. After 16 weeks the absorption was doubled, while the strength dropped to about 70 percent of the controls. Storage of *P. abies* under water for three years resulted in a reduction in impact strength, bending strength and compression strength of 10 to 15 percent (Adolf, Gerstetter and Liese 1972). The reductions in strength were restricted to the outer part of the sapwood which was infected by bacteria.

Examining the effect of bacteria on the strength characteristics of infected wood, Greaves (1973) used 20 wood inhabiting bacteria and *Eucalyptus regnans* and *Pinus radiata*. He found that all bacteria produced small, but highly significant weight loss, and strength loss could also be found. Greaves considered the mechanism of attack to be at the ultrastructural level, affecting the regular

alignment of cellulose microfibrils within the wall lamellae. That the modulus of elasticity in pine was unaffected by the bacteria he attributes to the pine cell walls being more resistant to enzyme attack than *Eucalyptus*. However eight bacterial species affected the modulus of rupture, a possible reflection on cell wall chemistry.

Thus experimental work so far has shown that while short term storage of wood under water does not seriously affect the strength properties of wood, long term storage apparently does. Various workers have indicated the possible macimum length of time storage could be safely carried on with respect to eventual end use of the timber. In this study the gross strength characteristics of *Pinus radiata* were examined after three and four years of sprinkler storage; the end use considered was building construction.

## 4:1 STATIC BENDING

In the static bending test a specimen 300 x 20 x 20 mm is supported over a span of 280 mm on platforms carried on roller bearings. The load is applied to the centre of the beam, the loading head descending at a constant speed of 0.11 mm/s (Lavers 1969). The load is applied tangentially to the annual rings. Load-deflection diagrams are recorded for all tests.

The strength characteristics determined were modulus of elasticity, modulus of rupture and work to maximum load. The bending strength or modulus of rupture is worked out using Navier's formula which is based on the assumption that the stresses are distributed linearly and symmetrically over

the cross section of the bent beam. This formula is strictly applicable for elastic deformation of beams up to the proportional limit, but it can be used for *comparative* studies beyond the proportional limit where it describes behaviour approximately. The numerical value for the modulus of rupture is obtained as follows:

$$\sigma = \frac{3P_{max}\, L}{2\, b.\, h^2}$$

Where $P_{max}$ = force applied at the moment of failure, L = span between supports, b = breadth of the beam and h = height of the beam.

Modulus of elasticity expresses the relationship between stress and strain and is of importance in determining the deflection of a beam under load. Elasticity concerns deformations produced in a solid body under low stresses. These are completely recovered after unloading (Kollmann and Cote 1968). The elastic properties are charasteristic for solid bodies below a certain limit of stress; above this limit plastic deformations or failure will occur. This relationship between deformations (strains) and stress within certain stress limits is of importance. The following exponential law is valid for all building materials except marbel and rubber.

$$\xi = \alpha \cdot \sigma^n$$

where $\xi = \frac{\text{elongation}}{\text{original length}}$ ; $\sigma$ = stress, and $\alpha$ and n are material constants.

For wood, experiments have shown n = 1, therefore Hooke's law states that the strain $\xi$ is proportional to the stress $\sigma$ :

$$\xi = \alpha \cdot \sigma$$

where $\varepsilon / \sigma$ is the compliance (strain per unit stress). Normally the reciprocal value E (modulus of elasticity or Young's modulus) is used:

$$^{1}/\alpha = E$$

The modulus of elasticity can be calculated from the initial part of the load-deflections curve:

$$E_b = \frac{P_{PL} \cdot L^3}{4.f.b.h^3}$$

where $E_b$ = modulus of elasticity in bending, $P_{PL}$ = force at proportional limit, L = span between supports, f = deflection of the midpoint of the beam at the proportional limit, b = breadth of the beam and h = height of the beam. The value for $E_b$ obtained using this equation is slightly less than the true value since the deflection is not only caused by axial strains but also by longitudinal shear in three point loading.

Work to maximum load provides a measure of the toughness of timber under bending stress and is determined from the area under the load-deflection curve to the point of maximum load. This is calculated by finding the area under the load-deflection curve and dividing the result into the span portion of the beam:

$$W_{max} = \int P.df = n\ P_{max}.f_{max}$$

where $P_{max}$ = maximum load and $f_{max}$ = maximum deflection.

4:1:1 Sampling after Three Years' Sprinkling

In the present study a sampling of the Balmoral logpile was made after three years of sprinkling using a

nested sampling strategy as outlined in Appendix C1. One hundred and twenty-eight samples were taken from logpiles 1 and 3 (Appendix A1). The samples were kiln dried to below 20 percent moisture content; 20 x 20 x 300 mm samples were machined and these sticks were stored at 25$^{o}$C and 60 percent relative humidity for four months to stabilize. Following this period a static bending test was done, as outlined earlier using an Instron Model 1195. The results were used to calculate modulus of rupture, modulus of elasticity and work to maximum load.

Analysis of the results summarised in Appendix D1 indicates that nothing was gained from the use of a nested design for sampling. There was no significant difference between samples from the outer and inner sapwood nor was there a difference within the logpile rows (Appendix D1). Therefore a random sample could have been taken from any position in a row of logs. In fact the percentage variance components for position of log within row and depth of sample within position of log accounted for 5.1702 and 0.0169 respectively. The overriding influence in this case could well be the sprinkling.

There was a significant difference between row 1 and row 3 however. This level accounted for 29.9475 and 36.9324 percent of the variance components and was significant at the 5 percent level. A further breakdown of the results, Table 4:1, demonstrates that the mean modulus of rupture was lower for row 1. It was expected that if any difference was to be found it would be in the outer logs as these would be exposed to greater environmental changes. No difference

could be measured between the top logs and bottom logs of a row so the mechanism may not be completely environmental. Row 1 was the first to be laid down, its height was slightly more than row 3 and it was separated by 4 m from row 2, while row 3 has less than 2 m separating it from row 2 and row 4.

Table 4:1

Mean Values of Elasticity, Rupture and Work to Maximum Load of Samples taken from Rows One and Three

| | Mean ($10^6$ N/m$^2$) | Standard Deviation ($10^6$ N/m$^2$) |
|---|---|---|
| Modulus of Rupture Row 1 and row 3 | 71.25 | 19.31 |
| Modulus of Elasticity Row 1 and row 3 | 7098.50 | 2204.30 |
| Work to Maximum Load Row 1 and row 3 | 0.019 | 0.016 |
| Modulus of Rupture Row 1 | 66.75 | 19.08 |
| Modulus of Rupture Row 3 | 75.13 | 17.96 |

Since all rows had similar levels of watering this is precluded. The most likely explanation is the position of row 1 as the outer row and the separation from the rest of the logpile. Previous work with large sprinkled logpiles (Liese and Karstedt 1971; Arnold *et al.* 1976; Peek and Liese 1976) has emphasised the need for a compact logpile.

### 4:1:2 Sample after Four Years' Sprinkling

One year later, four years after sprinkling commenced, a second sampling was conducted. All samples taken were treated similarly to that outlined earlier. However this

time an assumption was made, that there was no difference within the logpile. Although not strictly true as discussed above, the purpose of this sampling was to determine to what extent the strength characteristics as measured by static bending had differed from unsprinkled. As there were no data available on the strength of the wood when it went into the logpile, fresh timber was sampled, as in chapter III. Trees of similar age and from the outer edges of one of the compartments that logpile trees came from were sampled for comparison.

Modulus of Rupture, Modulus of Elasticity and Work to Maximum Load were determined and the results obtained were defined as the dependent variable in a regression analysis; density was made the independent variable and correlations were sought for all combinations. A stepwise multiple regression was used to fit the data to the model:

$$Y = B_0 + B_1X_1 + B_2X_2^2 + B_3X_3^3$$

where $B_0, B_1, B_2, B_3$ are constants

Tests were conducted with the load applied tangential to the annual rings (German Standard Specification DIN 52186) but with the applied load consisting of a constant crosshead speed, rather than a set loading rate per time.

Treating the three parameters separately the difference of the Modulus of Rupture between sprinkled and unsprinkled was significant (Appendix D2). The probability 0.0027 for F = 9.26 means we reject the null hypothesis of no difference and assume a difference taking P = 0.01 as the cutoff point. The sprinkled wood had a higher mean modulus

of rupture 84.6 x $10^6$ $N/m^2$ than the unsprinkled with a mean of 76.5 x $10^6$ $N/m^2$ (Table 4:2). For modulus of elasticity no significant difference was found between sprinkled and unsprinkled wood at the one percent level, the mean value for sprinkled being 9089.4 x $10^6$ $N/m^2$ and for unsprinkled 8508.7 x $10^6$ $N/m^2$. Similarly Work to Maximum Load did not show any significant difference between sprinkled and unsprinkled wood. The mean value for sprinkled wood was 0.134 x $10^6$ $Nm/m^3$ and for unsprinkled wood 0.145 x $10^6$ $Nm/m^3$.

Table 4:2

Mean values of Static Bending Tests on Balmoral Wood Sprinkled for Four Years and Not Sprinkled

| | Sprinkled | | Not Sprinkled | |
|---|---|---|---|---|
| | Mean | Standard Deviation | Mean | Standard Deviation |
| | ($10^6$ $Nm/^2$) | | ($10^6$ $N/m^2$) | |
| Modulus of Rupture | 84.56 | 15.59 | 76.58 | 15.11 |
| Modulus of Elasticity | 9089.4 | 2111.2 | 8508.7 | 1722.7 |
| Work to Maximum Load | 0.134 | 0.073 | 0.144 | 0.010 |

Modulus of Rupture is the equivalent stress in the extreme fibres of the specimen at the point of failure (Lavers 1969). Stress is simply the load per unit area Gordon 1968). So modulus of rupture is a measure of the ultimate bending strength of the timber. As both the sprinkled and unsprinkled wood were treated similarly from point of sample the higher bending strength of the sprinkled wood can be explained only by some difference in the wood's structure. As the wood was not end matched samples but only random selections some of the difference could be due to

chance. However the highly significant difference recorded suggests that the difference is large as well as real. Kollmann and Cote (1968) in discussing the relationship between bending strength and density quote two different workers who obtained different relationships, one linear and one curvilinear. Their explanation for the difference was the higher amount of resin in one species (pine) in relation to the other (ash) which, they claim, increases the density but lowers the cohesion.

A regression analysis of bending strength and density (Appendix D3) shows that a linear relationship is a reasonable approximation. As before there is a difference between sprinkled and unsprinkled but the slopes of the two lines are not significantly different. The difference between the two lines represents $16.36 \times 10^6$ $N/m^2$ which if the assumption of higher density through higher resin content signifies a lowering of cohesion then the difference of density between sprinkled and unsprinkled of 0.068 $g/cm^3$ at any one bending strength is due to a loss of material in the sprinkled wood. This material lost would be extractives and materials consumed by organisms within the wood.

The modulus of elasticity showed a significant difference between sprinkled and unsprinkled at $p = 0.05$ but not at $p = 0.01$. Modulus of elasticity expresses the relation between stress and strain and is of importance in determining the deflection of a beam under load. Once again the relationship between modulus of elasticity and density appears to be essentially a linear relation (Appendix D4). That the difference between sprinkled and unsprinkled is only

significant at $p = 0.05$ suggests that whatever damage has occurred to the sprinkled wood, and it has already been demonstrated that changes have occurred, the damage to the cellulose fibrils does not appear large. As outlined above elasticity measures the deformations at which a beam will recover after unloading. The fibrils in the wood must play an important part in this form of deformation, as they have a high tensile strength (Stamm 1946).

However as shown in Appendix D5 there was no demonstrable difference in the densities of sprinkled and unsprinkled wood. This could mean that the method of measuring density, oven dry weight over volume displacement, was not precise enough to show any loss of material. Some changes to wood structure have been demonstrated in Chapter II. Therefore the change that has occurred to cause a difference in modulus of rupture but only a small difference in elasticity and no difference in work to maximum load, must be of a low order.

The properties of static bending can be sensitive to changes in wood structure (fracture patterns discussed later is an example) but are not the only measures which can be made. The hardness of the sprinkled and unsprinkled wood was also determined as this is another parameter that could have been affected.

## 4:2 HARDNESS

The hardness of all the wood tested in 4:1 was determined using the relationship

$$\text{Hardness} = \frac{L}{A}$$

where L = load and A = projected area.

The test used wedges as the indenting tool, the procedure outlined by Doyle (1980).

Evaluation of hardness was made to determine whether sprinkling had affected any other properties of the wood. Results obtained (Appendix D6) show that no difference could be detected between sprinkled and unsprinkled wood. A relationship was established between density and hardness although a linear function did not fit well. Using the squared terms of hardness with density gave a difference in slope and a difference between sprinkled and unsprinkled wood. Hardness has been affected by sprinkling but the measure is not as sensitive as modulus of rupture.

## 4:3 FRACTURE PATTERNS

While testing by static bending, a difference in fracture patterns between sprinkled and unsprinkled wood was noted. It appeared that on failure, the break in the sprinkled wood appeared brash. Brashness is often related to degradation of wood but is more often related specifically to fungal degradation.

To attempt to quantify this observation five categories of break characteristic were enumerated (see ASTM D143).

a) Simple tension

b) Cross grain tension

c) Splintering tension

d) Brash tension

e) Compression

The term "cross grain" is considered to include all deviations of grain from the direction of the longitudinal axis of the specimen.

The result tabulated in Table 4:3 presents a striking difference between sprinkled and unsprinkled wood.

Table 4:3

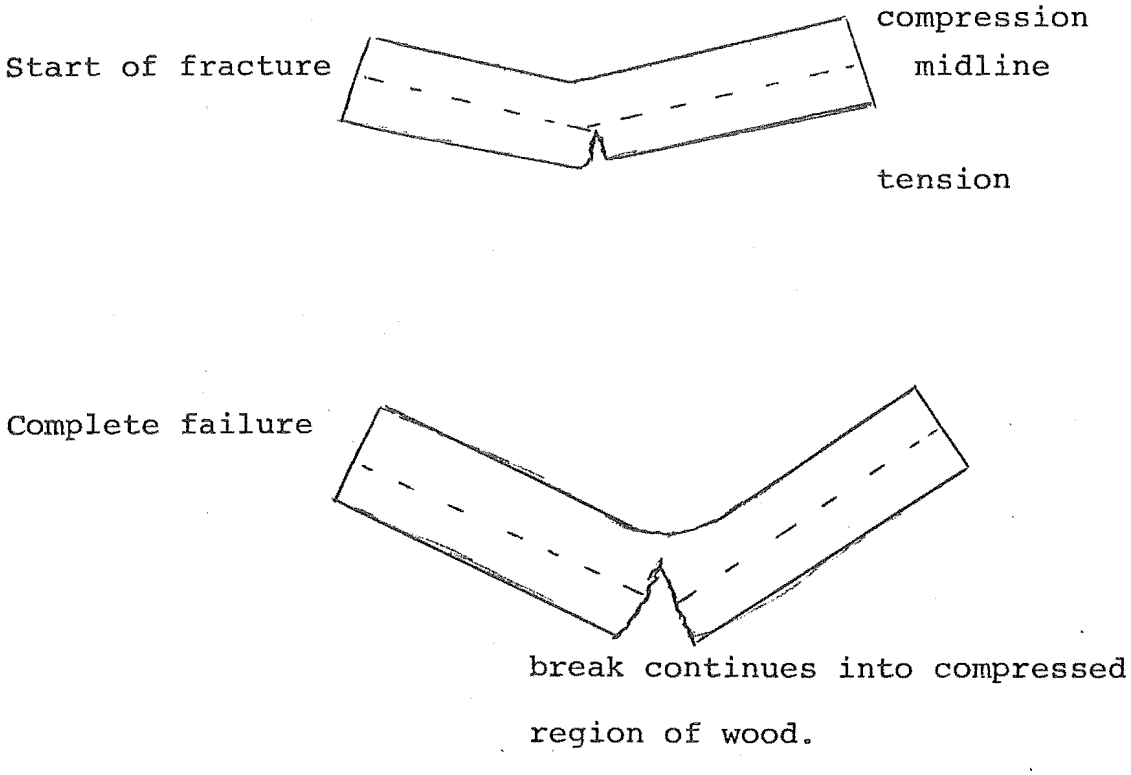
Failure in Static Bending

| Category | Sprinkled % failed | Unsprinkled % failed |
|---|---|---|
| a | 49 | 68 |
| b | 8 | 10 |
| c | 0 | 4 |
| d | 39 | 16 |
| e | 4 | 2 |
| Total tested | 100 | 50 |

More brash failures were recorded for sprinkled wood than from unsprinkled wood. Of these brash failures recorded in the sprinkled wood (39 percent) almost half, (48 percent) showed a pattern of break which appeared to be simple tension failure except that the tension break below the midline continued up into the wood under compression above the midline so that the complete failure was recorded as brash. This pattern of failure did not occur in the fresh wood (see Figure 4:1).

Figure 4:1

A typical Brash Fracture Pattern




The increase of brash failure under static bending in the sprinkled wood adds weight to the theory concerning the observations made for bending strength, whereby removal of intercellulosic material allows the cellulose fibres to orientate more. Cellulose fibres fail as a result of fibrils or microfibrils sliding and pulling one from another (Stamm 1946). Fibres will elongate or creep under a constant tensile stress. The increase in brash failures and the high modulus of rupture in sprinkled wood are therefore both measures of this same phenomenon. The sprinkled wood did not show an increase in Young's modulus as this is a measure of elasticity recovery, but a loss of inter cellulose material may have allowed the cellulose fibres to move further before failure and thus a greater

modulus of rupture. However at failure the stresses applied were greater than for unsprinkled wood and so the stored energy caused a break which occurred suddenly as the cellulose fibres parted, giving a brash fracture pattern.

This is very important if the timber is to be considered for applications where a permanent load or dead load is applied. Under these conditions fibres will break at loads of considerably lower magnitude than the normal rapidly applied breaking load. The amount of creep could be unacceptable in the sprinkled wood under these conditions.

# CHAPTER V

## PRACTICAL ASPECTS OF SPRINKLING

Sprinkling logs with water maintains a moisture content within the logs at least equal to the moisture content of fresh green wood. Despite doubts about the mechanisms involved, experience has shown that if the moisture content can be maintained above 100 percent then the stored wood remains sound (Peek and Liese 1974).

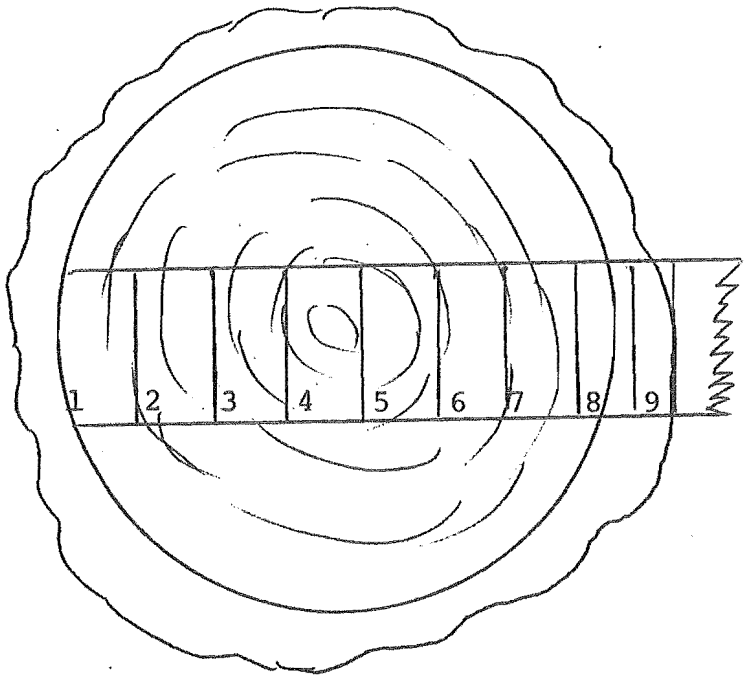
The moisture content achieved by sprinklers appears to vary from 100 percent (Liese and Karstedt 1971) to over 150 percent (Miller and Swan 1980) in sapwood, the variation probably being a reflection of the different species. Miller and Swan (1980) investigated different on-off sprinkler cycles. They found that in the pile that had a cycle of six minutes on and 34 minutes off, moisture content in the sapwood of *Pinus ponderosa* fell below 100 percent and sap stain was recorded. All other cycles gave an adequate protection through maintaining a moisture content over 100 percent.

This figure of 100 percent appears to give the manager of a sprinkled logpile a practical measure to apply for control of the logpile; moreover it is a measure easily made. To determine whether this figure is also applicable to *Pinus radiata* a measure of the moisture content of the Balmoral logpile was made, the results being compared with the microbial samplings described in Chapter VII.

## 5:1 MOISTURE CONTENT

A check was made on the moisture content of the Balmoral logpile after four years' sprinkling. Random samples were taken through rows one and two (Appendix A1) in an attempt to gain an idea of the mean moisture content. Logs were selected at random, the first 600 mm of log was sawn off and discarded and a grid was placed on the cut end of the log ensuring that it ran through the pith region (figure 5:1). One sector of the grid was chosen randomly

Figure 5:1




and a piece approximately 30 x 30 x 50 mm was removed, sealed in polythene, chilled and removed for measurements of moisture content and density. Fresh felled *Pinus radiata* of a similar age was treated in a similar manner.

Moisture content was found to be higher in both the sapwood and heartwood of the sprinkled wood (Table 5:1 and Appendix E1). Clifton (1978) recorded a similar sapwood

moisture content in samples he took from this logpile after one years' sprinkling, but a higher moisture content of up to 160 percent after two years' sprinkling. The moisture content found in this study after four years' sprinkling is lower than the level reported after two years' sprinkling.

Table 5:1

Moisture Content and Density of Balmoral Fresh and Sprinkled Wood

| | Sprinkled Wood | | | | Unsprinkled Wood | | | |
|---|---|---|---|---|---|---|---|---|
| | Sapwood | | Heartwood | | Sapwood | | Heartwood | |
| | mean | SD | mean | SD | mean | SD | mean | SD |
| Density ($kg/m^3$) | 422.3 | 40.9 | 382.5 | 45.7 | 489.7 | 39.2 | 445.9 | 56.8 |
| Moisture Content % | 131.1 | 24.7 | 54.4 | 14.3 | 110.9 | 10.4 | 38.9 | 10.5 |
| Saturation % | 85.1 | 11.8 | 46.2 | 6.6 | 86.7 | 4.5 | 47.1 | 8.1 |

The moisture content of 134 percent was found to be significantly higher than that of fresh wood which was 111 percent at the time of sampling.

The likeliest explanation of the disparity between results of this study and Clifton's (1978) is the trouble experienced in 1979 and 1980 with failing rubber connectors in the water lines and failing sprinkler heads. However, that this may be linked to the general degrade of the logs cannot be dismissed. Huang, Sarkanen and Johanson (1977) have shown that the diffusivity of dissolved oxygen in water saturated softwood increased with increasing delignification, while Petty and Preston (1969) found the portion of air trapped in Sitka spruce increased with increasing moisture content between 0 percent and 30 percent M.C. before levelling

off. If these two were acting together in the Balmoral logpile it could well be that the loss of cell contents and increasing moisture content might result in an increase in trapped gas which could effectively restrict the total achieveable moisture content as degradation progressed. However this is a supposition and needs the support of further research to understand the mechanisms of water storage.

Work presented in Chapter VII indicates a general rise in frequency of isolations of fungi especially after four years of sprinkling. Whether this can be explained by the lowering moisture content cannot be shown from this work although it appears likely.

This study has shown no significant difference in density between the sprinkled and fresh sapwood. Work reported earlier also did not show a difference although sapwood and heartwood were not considered separately.

The percentage saturation in sprinkled sapwood was significantly greater than that for fresh. This is important when freighting the logs for any distance. The increased weight means a smaller payload and hence increased cartage costs. Cartage costs are increasingly important when considering the total costings of a particular proposal. The additional costs incurred in transporting unwanted water coupled with the costs of sprinkling (pumps, sprays, lines, power, etc.) must increase the cost of the finished product considerably.

The question also arises as to the condition of the finished product. No colour difference could be detected

when three year sprinkled logs were being sawn at the same time as fresh wood. Clifton (1978) reports a similar finding after the logs had been sprinkled for two years. However Haslett (1980) reports that there was some colour change in wood that was kiln dried after sprinkling for two years. After four years' sprinkling some sawmills reported that a colour developed after sawing. This colour, they claimed, was unacceptable to the industry. However the 'coloured wood' once air dried showed no difference in colour from fresh wood in all samples taken. This was also noted by Clifton (pers. comm.).

A study was also made of the susceptibility of sprinkled wood to sapstain. This was to determine whether extra precaution need to be taken when air drying sprinkled wood.

## 5:2 SUSCEPTIBILITY TO SAPSTAIN

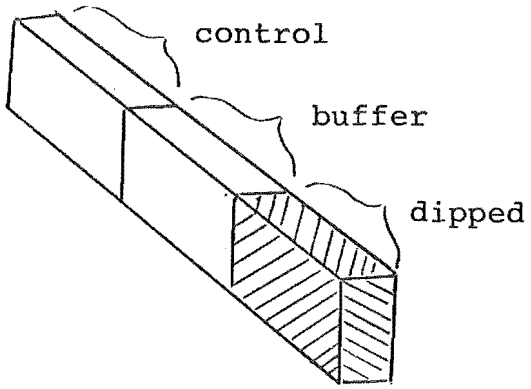
Samples measuring 300 x 75 x 25 mm were cut from Balmoral wood of the same age:

a) sprinkled for four years (24 pieces)

b) not sprinkled (12 pieces)

Within twentyfour hours of cutting, each piece was divided into 100 mm sections and one section of each piece was dipped into a concentration of a sapstain preventative (Captafol) to one third of the wood's total length (Figure 5:2) after the method of Butcher (pers. comm.).

Figure 5:2




Three different concentrations were used: 300 g/ℓ, 150 g/ℓ, and 75 g/ℓ. After dipping, all wood pieces were allowed to drain dry before being fillet stacked in a tray, sprayed with a mixed inoculum of sapstain organisms (see Appendix E2), and covered with black polythene. After eight weeks of storage at room temperature during which a high relative humidity was maintained through water in the bottom of the tray, the stack was dismantled and all the pieces rated using the growth on the control as one extreme and clear wood as the other. A scale of 1 to 5 was employed with 5 being complete coverage. The scale for rating the amount of infection was as follows:

Score 1 = no infection

2 = stain patches cover 25 percent of total wood surface

3 = stain patches cover 50 percent of total wood surface

4 = stain patches cover 75 percent of total wood surface

5 = complete coverage, no clear patches of wood, all being coloured.

Table 5:2

Mean Level of Sapstain Infection after Eight Weeks

| Captafol Concentration | Fresh Wood | Sprinkled Wood | F |
|---|---|---|---|
| 300 g/ℓ | 1.5 ± 1 | 2.4 ± 0.5 | 3.5 |
| 150 g/ℓ | 1.5 ± 0.5 | 2.3 ± 0.75 | 0.44 |
| 75 g/ℓ | 2.5 ± 1 | 3.2 ± 0.98 | 1.23 |

The sprinkled wood was at least as susceptible to infection by sapstain organisms (Table 5:2) as fresh wood. The most common organism found was *Trichoderma*. Some of the sprinkled wood also showed a red colouration on the outside although no stain penetration was observed in any of the pieces.

The results are a little surprising in that it would be expected that the fresh wood would offer more simple carbohydrates for initial colonization by fungi. Nevertheless the result is important as it implies that care needs to be exercised when stack drying sprinkled *Pinus radiata*. At the recommended use concentration of 300 g/ℓ Captafol, an unacceptable result was obtained when compared with fresh wood. Although this test is severe in that no drying is allowed (so favouring rapid growth of the sapstain fungi) over the period of drying taken in a large stack, similar moisture conditions could be encountered. Agreement between the replicates was close, each category rating similarly with the means fairly representing the overall result. However the rating scale is subjective and this must be allowed for in interpretation.

# PART II

# BIOLOGICAL ASPECTS OF SPRINKLING WOOD

# CHAPTER VI

# MICRO-ORGANISMS IN WOOD

## 6:1 FUNGI ASSOCIATED WITH WOOD DECAY

A number of species of fungi live in or on wood. Käärik (1974) grouped wood inhabiting micro-organisms into the following major categories according to their enzymatic activity:

1) Fungi living on dead cell contents: no enzymatic degradation of cell walls:
   - (a) moulds;
   - (b) blue stain fungi;

2) Organisms capable of enzymatic breakdown of cell wall components:
   - i) with a limited capability to degrade:
     - (c) bacteria;
     - (d) soft rot fungi;
   - ii) decay fungi with a high capability to degrade:
     - (e) brown rot fungi;
     - (f) white rot fungi;

Garrett (1963) outlined a generalized scheme for fungal succession on woody substances in or on soil, as did Hudson (1968):

Senescent tissue

| stage 1a | stage 1 |
|---|---|
| weak parasites | primary saprophytic, sugar fungi |

Dead tissue

| stage 2 | stage 3 |
|---|---|
| cellulose decomposers; secondary saprophytic sugar fungi | lignin decomposers; |

This scheme starts with the organisms colonising the living tissue. However in timber there is no selective influence as may be exerted by a living tree, only organisms competing amongst themselves for the available substrate. Butcher (1968) followed the succession of fungi colonising untreated stakes of *Pinus radiata* sapwood. Above ground the colonisation proceeded no further than blue stain fungi to moulds while at groundline and below the succession was primary moulds to soft rot fungi to secondary moulds to basidiomycetes. Bannerjee and Levy (1971) made a similar observation for above ground succession on birch wood.

Wood stored in logpiles would be expected to have a succession similar to that found in above ground studies. Buchanan (1940) following the deterioration on wind felled trees found that the succession did proceed to basidiomycetes but was very slow, taking over two years. *Fomes pinicola* and *F applanatus* were responsible for most of the decay. *Pinus radiata* pulpwood chips stored in outside piles in New Zealand were found to have basidiomycete or soft rot decay within six months (Butcher and Howard 1968).

*Peniophora gigantea* and *Odontia bicolor* were the dominant wood rotting fungi and *Helicosporium aureum* and *Helicomyces sp* the main soft rot agents.

Rough round wood with undamaged or partially damaged bark stored in log decks, or in ponds for six months in Poland was infected by blue stain fungi (Tarocinski and Zielinski 1977). *Alternaria tenuis, Aureobasidium pullulans, Trichoderma koningii,* and *T. lignorum* were found in both the above situations very frequently with twentysix different fungi in total being isolated.

At the commencement of this project after the logs had been stored for three years two random samplings, one month apart were conducted using a nested sampling design (Appendices A1, C1). One hundred samples (increment cores) were taken at each sampling and from each core three discs were cut out and plated on malt agar for fungal isolation as detailed in Appendix F1. Of the three discs cut from each core one was from the outer sapwood and one from the outer heartwood. The results showed a very low level of fungal infection with all isolations except one confined to the outer sapwood (Table 6:1). All isolations made were from the Moniliaceae and Tuberculariaceae, placing them within the first stage of the successions described by Käärik (1974), Garrett (1963), and Hudson (1968). The fact that very few fungi were isolated after three years of sprinkling and that all were primary saprophytes, indicates that no succession was found; rather these fungi represent the first stage in the succession mentioned earlier.

Table 6:1

Isolations from the Balmoral Logpile after Three Years' Sprinkling

| | Sample 1 | | | Sample 2 (one month later) | | |
|---|---|---|---|---|---|---|
| | Outer sap | Inner sap | Outer heart | Outer sap | Inner sap | Outer heart |
| Trichoderma | 5 | 0 | 0 | 6 | 0 | 0 |
| Fusarium | 2 | 0 | 0 | 2 | 0 | 0 |
| Verticillium | 1 | 0 | 0 | 1 | 0 | 0 |
| Penicillium | 1 | 1 | 0 | 2 | 0 | 0 |
| Isolations | 9 | 1 | 0 | 11 | 0 | 0 |
| Total samples | 100 | 100 | 100 | 100 | 100 | 100 |

Two further samplings were made to check the level of fungal infection on the Balmoral logpile. One after three and a half years' sprinkling consisted of thirty random samples. This sampling returned one isolate of *Fusarium* indicating no change from earlier samplings.

The final sampling of the Balmoral logpile was made only from rows 1 and 2 (Appendix A1) because of the partial dismantling and sale of the logpile. This sampling was conducted four years and two months after sprinkling started. Twenty cores were taken, ten from logs near the top of the rows and ten from the bottom logs of the rows. Two discs were taken from each core, one from the outer heartwood and one from the outer sapwood. Isolation methods were as outlined in Appendix F1 and the results are shown in Table 6:2.

Table 6:2

Fungi isolated from the Balmoral Logpile after Four Years' and Two Months' Sprinkling

| | Top Logs | | Bottom Logs | |
|---|---|---|---|---|
| | sap | heart | sap | heart |
| Cephalosporium | 1 | 0 | 3 | 0 |
| Gliocladium | 2 | 0 | 0 | 0 |
| Goidanichiella | 1 | 0 | 0 | 0 |
| Moeszia | 2 | 1 | 0 | 0 |
| Trichoderma | 1 | 0 | 1 | 0 |
| Verticillium | 0 | 0 | 1 | 0 |
| Fusarium | 1 | 1 | 0 | 1 |
| Basidiomycetes | 2 | 0 | 3 | 1 |
| Total isolations | 11 | 2 | 8 | 4 |
| Total samples | 10 | 10 | 10 | 10 |

Once again the majority of isolations were from the Moniliaceae, however there were some potential soft rotting organisms as well as some basidiomycetes suggesting some change from the colonization pattern of earlier samplings. The number of positive isolations also greatly increased indicating an increase in fungal activity. This could have resulted from more intermittent sprayings, as some of the logpile had been sold and the sprinklers were turned off while extraction took place. This happened at the onset of warmer weather which would cause rapid drying while the water was off. However at the same time, sawmillers handling the timber showed concern at the occasional log

found with fungal degrade. This degrade was far greater than could be explained by the degree of fungal activity indicated by the samplings. Investigations indicated that some logs were stored without sprinkling for up to four weeks before milling (Dougherty *pers. com.*) and this could account for logs with initial fungal activity showing a large increase in fungal degrade, although sampling error could also account for it.

Further fungal isolations were made from an experimental small scale logpile and these will be described later in this chapter.

## 6:2 BACTERIA ASSOCIATED WITH WOOD DECAY

As noted in Chapter II bacteria have been known to be associated with wood, particularly wood decay, for a long time. They appear to be associated primarily with parenchymatous tissues, regardless of the type of wood.

Bacterial attack or the mere presence of bacteria in wood appears to be closely associated with the amount of water in the material. Situations where the wood is saturated with water (when stored in log ponds or under sprays, in the ground, in rivers or sea) create the conditions which encourage the colonization of that wood by bacteria. Living as well as dead portions of wood can be colonized by bacteria.

### 6:2:1 Bacteria in living trees

Hartley, Davidson and Crandall (1961) isolated bacteria from living wood and postulated that the condition known as wetwood was caused by bacteria. Schroeder and

Kozlik (1972) suggest the converse and consider wetwood as the reason for the presence of bacteria. Whatever the sequence bacteria have been isolated from living trees, and especially from wetwood.

Two genera of bacteria and a yeast were found by Knutson (1973). Expressed sap from sapwood of Aspen trees yielded eighty colonies of *Bacillus* per ml and 590 colonies of *Erwinia* per ml. Aho *et al.* (1974) obtained 1200 bacterial isolations from living White Fir, purified 572 isolates and found that 40 percent were aerobic pseudomonads, 2 percent *Bacillus sp.* and 58 percent were gram negative, fermentative bacteria. Most of the latter were classified as *Enterobacter*.

6:2:2 Bacteria in wood

*Aerobacter, Bacillus* (especially *B. polymyxa* and *B. subtilis*) and *Pseudomonas sp.* were isolated by Knuth (1964) from Ponderosa pine. *B. polymyxa* has been found by several workers (Greaves 1971; MacPeak 1963; Ellwood and Ecklund 1959) in pine. A number of strains of bacteria were tested for decay capability by Schmidt (1978) who found *B. polymyxa* one of only two to affect cell wall content, and this only after some removal of lignin. Schmidt concluded that the more lignin removed beforehand, the better the decay.

A review of the types of bacteria found in wood has been given by Rossell, Abbot and Levy (1973) and it is clear that numerous kinds of bacteria have been isolated from wood. For instance Boutelje and Göransson (1971) isolated *Escherichia coli, Cellvibrio,* and species of *Bacillus, Pseudomonas, Arthrobacter, Flavobacterium, Cellulomonas, Plectridium,* and *Clostridium.*

Boutelje and Göransson (1971) studied the wood from piles below the ground water table and found that non cellulolytic bacteria could penetrate wood to a considerable depth. These bacteria were not harmful to the cellulose or wood, but the authors suggested that they played a subsidiary role in decay by supplying active wood destroying bacteria with growth factors. Schmidt and Dietrichs (1976) isolated bacteria from spruce (*Picea abies*) and pine (*Pinus sylvestris*) and tested the isolates for their ability to utilize various wood components as their sole carbon source. Of the 150 strains tested 23 percent were able to degrade pectin, 17 percent xylan, 10 percent carboxymethyl-celluloses, 9 percent holocellulose and 6 percent a-cellulose. Only two strains were capable of degrading untreated wood chips. One of these two strains was derived from sprayed pine and was active against spruce chips while the other was a culture of *Cellulomonas* and was active against beech (*Fagus sylvatica*) chips. The conclusions drawn were that degradation of the wood components occurs most frequently under aerobic conditions and that bacteria from sprayed wood are less active than those isolated from other environments.

6:2:3 Bacteria in water soaked wood

As mentioned already, Boutelje and Göransson (1971) isolated large numbers of organisms from pine piles, finding both aerobic and anaerobic bacteria. Harmsen and Nissen (1965) also isolated micro-organisms from old foundation piles, in particular actinomycetes and *Bacillus sp.* Greaves and Levy (1968) found evidence of bacterial degradation in mine timbers up to 100 years old.

However the interest in bacteria associated with water soaked wood stemmed from the work of Ellwood and Ecklund (1959a and b) who were studying the reason for the high porosity developed by water stored ponderosa and sugar pine. As noted in Chapter II, the excess porosity developed because of depletion of the contents of the ray parenchyma. The causative organism was identified as *Bacillus polymyxa*, which was capable of attacking hemicellulose and pectin but not cellulose. This was the case for logs stored in ponds for one to several months. However, they found that decked, sprayed logs or logs in rapidly flowing water were not severely attacked.

MacPeak (1963) also identified *B.polymyxa* as the cause of excessive porosity in sapwood. *Desulphovibrio desulphuricans* was also isolated but MacPeak concluded that it was not actively involved in breakdown but was just associated with *B.polymyxa*. Knuth and McCoy (1962) found *Aerobacillus* and *Vibrio* in ponded logs while Lutz, Duncan and Scheffer (1966) isolated *Micrococcus* and *Pseudomonas spp. Bacillus polymyxa, B.subtilis, B.mesentericus, Staphylococcus spp., Clostridium omelianski* and *Flavobacterium pectinovorum* were isolated by Fogarty (1973). De Groot and Sachs (1976) found bacteria from the *Klebsiella-Aerobacter-Serratia* group in sprinkled southern pine, and there are reports from many others of bacteria isolated from water stored wood (e.g. Karnop 1972; Unligil 1971; Fogarty and Ward 1972; Ward and Fogarty 1973; Boutelje and Kiessling 1964; Berndt and Liese 1973; Liese and Karnop 1968; Feihl 1978).

Debarked spruce and scots pine were stored under water for 22 months by Liese and Karnop (1968) and periodically examined for the types of bacteria which attacked. The bacteria isolated were divided into four groups: coliform bacteria; other sporeless bacteria; aerobic and anaerobic spore producers. Some of the isolates exhibited pectinolytic activity and *Bacillus omelianskii* showed cellulolytic activity.

Karnop (1972) examined water stored scots pine in winter and summer. During the summer the bacteria isolated were mainly motile, gram negative rods and non motile, strongly granulated non sporing rods; vibrios and staphylococci were also found. In winter isolates included mainly gram negative, non sporeforming, motile bacteria which exhibited many characteristics similar to coliform organisms. Karnop had some difficulty in determining exactly what genera he was dealing with and concluded that they were coliform bacteria which had undergone changes as a result of environmental conditions:

> 'These strains must be considered ubiquitous, facultatively anaerobic bacteria whose physiology is different from that of the typical coli bacteria.'

Berndt and Liese (1973) had more success in identifying bacteria present in beech after six months' storage under water. Five groups of organisms were identified:

1) gram positive, non sporing bacteria; four species including *Staphylococcus epidermis, Arthrobacter sp., Kurthia sp.*

2) aerobic, sporulating bacteria; five species including *Bacillus cereus, B. megaterium, B. pumilus, B. brevis.*

3) gram negative, fermentative rods: four species including *Enterobacter spp.*

4) gram negative, oxidative rods: eight species including *Pseudomonas fluorescens, Pseudomonas spp.*

5) gram negative, non carbohydrate utilizing rods: five species including *Acetomonas sp., Flavobacterium spp., Alcaligenes faecalis.*

This present study commenced when the logs had been under sprinklers for three years. At this time an attempt was made to determine the level of the bacterial population, the sampling design being that set out in Appendix C1. One hundred increment cores were taken and from each core three discs were removed and the bacteria isolated using the method outlined in Appendix F1. Of the three discs cut from each core, one was from the outer sapwood, one from the inner sapwood and one from the outer heartwood.

The data presented in table 6:3 summarise the results obtained from the sampling; a more detailed presentation of results and analysis is given in Appendices F2 and F3. The sampling design attempted to determine whether position in the logpile was significant in terms of the distribution of the bacterial population. Of the six logpiles available for sampling two had an uneven distribution of logs (piles 4 and 5, Appendix A1) and so were not sampled. The four piles therefore were considered the highest level category in this study and clearly are fixed treatment effects and hence Model I.

Table 6:3

Summary of Bacteria and Yeasts isolated from Balmoral Logpile

| | Outer sap | Inner sap | Outer heart |
|---|---|---|---|
| Percentage samples yielding bacteria | 25.59 | 27.08 | 27.38 |
| Percentage isolates gram positive rods | 25.58 | 23.08 | 27.38 |
| Percentage isolates gram positive cocci | 8.14 | 2.2 | 11.96 |
| Percentage isolates gram negative rods | 40.70 | 35.14 | 36.96 |
| Percentage isolates yeasts | 37.21 | 39.56 | 22.82 |
| Total isolations | 100 | 100 | 100 |

The next level was the height in the pile from which the core was taken and this depended in part on the ability of the sampler to obtain a core. There were three general areas: that area which could be reached from the ground; the area which could be reached when standing on the top of the lower tier of logs in the pile (the piles were two-tiered, the top tier being logs 4 m in length, and the bottom tier being logs 8-12 m in length) and the top tier of logs. The third and lowest level category was the depths in the core that the isolations were made from. This was clearly

dependent on the diameter of the log sampled so these lower categories were tested as random treatment effects and hence Model II (Sokal and Rohlf 1969).

The analysis therefore became a mixed model nested ANOVA. From the computation of the results as summarised in Appendix F3 it would appear that position in the logpile is not significant. Almost all the variation is accounted for from within the core samples (96 percent) with very little being contributed by position in log or pile. This suggests that the bacteria found were distributed randomly throughout the sapwood and outer heartwood.

The high variance components reflect the degree of difference between isolation areas. Appendix F2 shows that some areas have a low population level of $10^1$ magnitude while others have greater than $10^3$. The suggestion is made that after sprinkling for three years the bacterial population has stabilized to the extent that this sampling is detailing the pattern of surviving pockets of bacteria rather than a dynamic successional population. If an active population had been encountered, less nil count samples would have been expected. On the other hand it could be argued that the succession is going forward but at a reduced rate. Ellwood and Ecklund (1959a) suggested this when they observed that wood stored under rapidly flowing water or sprinklers showed less attack than wood stored in ponds. Dunleavy and Fogarty (1971) following the numbers of bacteria in pond stored norway spruce and sitka spruce found that bacterial counts peaked after eleven weeks' storage.

Partial identification of some of the bacterial isolates showed a wide range of types. Pseudomonads, coryneforms, spore formers, staphlococci and coliform-like organisms were isolated. No type was isolated more frequently from one area than another. Table 6:3 shows the initial grouping of isolates according to gram reaction and cell morphology. The distribution of types relates well to other studies such as those of Schmidt and Dietrichs (1976) and Berndt and Liese (1973), and lends weight to the idea that there was a stable population of bacteria within the logpile at the time of sampling.

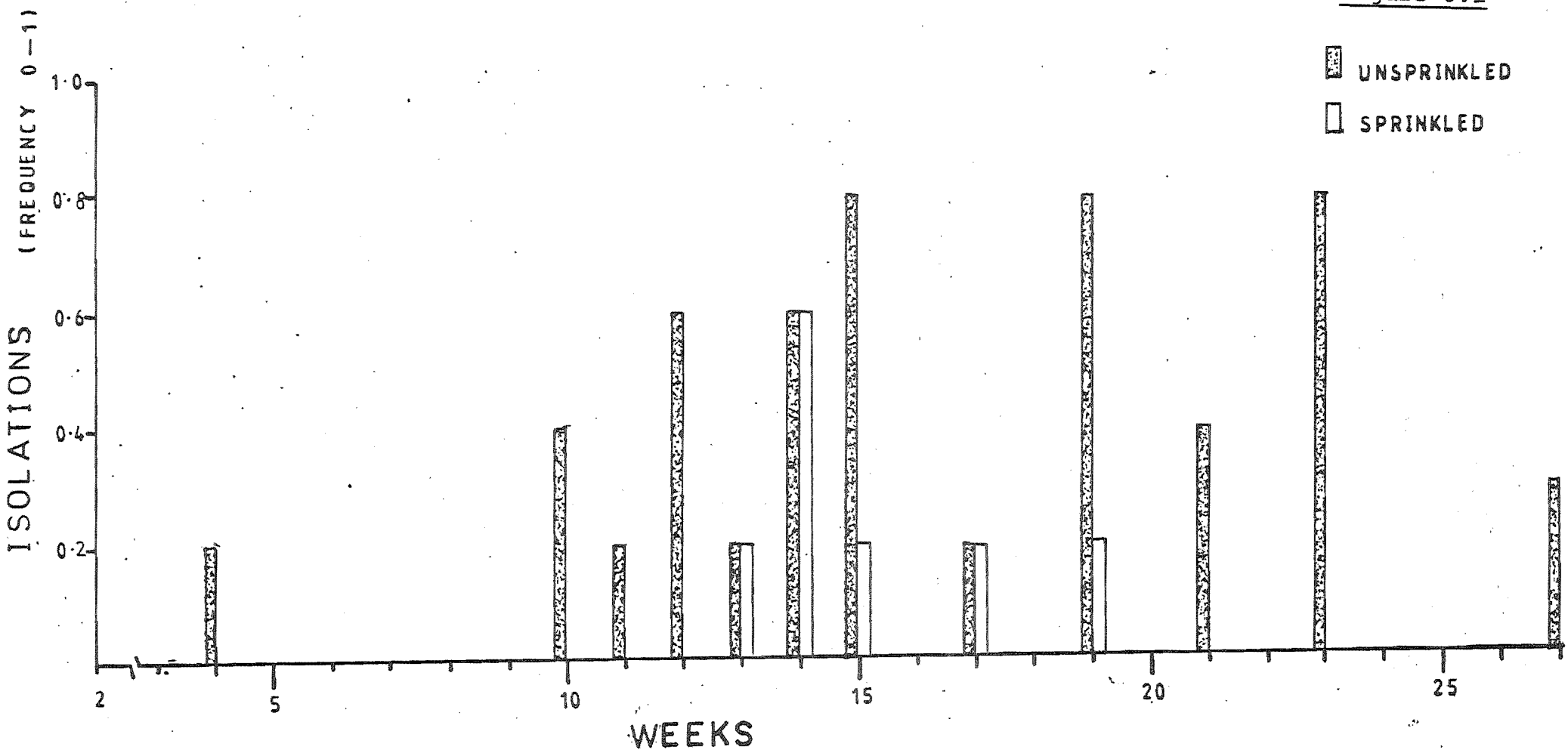
The next step taken in this study was to attempt to identify the early succession of bacteria within sprinkler stored *Pinus radiata*. As the Balmoral logpile was over two hours' travel from the laboratory and it was considered that frequent samplings would have to be made, a smaller logpile was constructed next to the laboratory. This allowed sampling whenever needed.

## 6:3 SMALL SCALE SPRINKLED LOGPILE

Small bolts of timber 2 m long and with an average diameter of 150 mm were formed into two piles, each pile supported on runners just above the ground. One pile contained $2m^3$ of *Pinus radiata* and was sprinkled continuously with a fine mist garden sprinkler while the other pile contained 1 $m^3$ of timber and was unsprinkled. Samples were taken at weekly intervals for fifteen weeks, the next four samples were at two week intervals and the last sample was four weeks later. Sampling was as outlined in Appendix C1,

# ISOLATIONS OF FUNGI FROM SMALL LOGPILE

Figure 6:1



five samples being taken from the unsprinkled pile and ten from the sprinkled pile: isolations were made only from the sapwood.

6:3:1 Fungal isolations

The isolations from both sprinkled and unsprinkled piles are summarised in Figure 6:1. As each sample disc was subdivided into six sticks and each stick placed on agar, it was felt more appropriate to represent the number of isolations by a 0-1 scale rather than total number. This gave a frequency of isolation rather than an absolute figure.

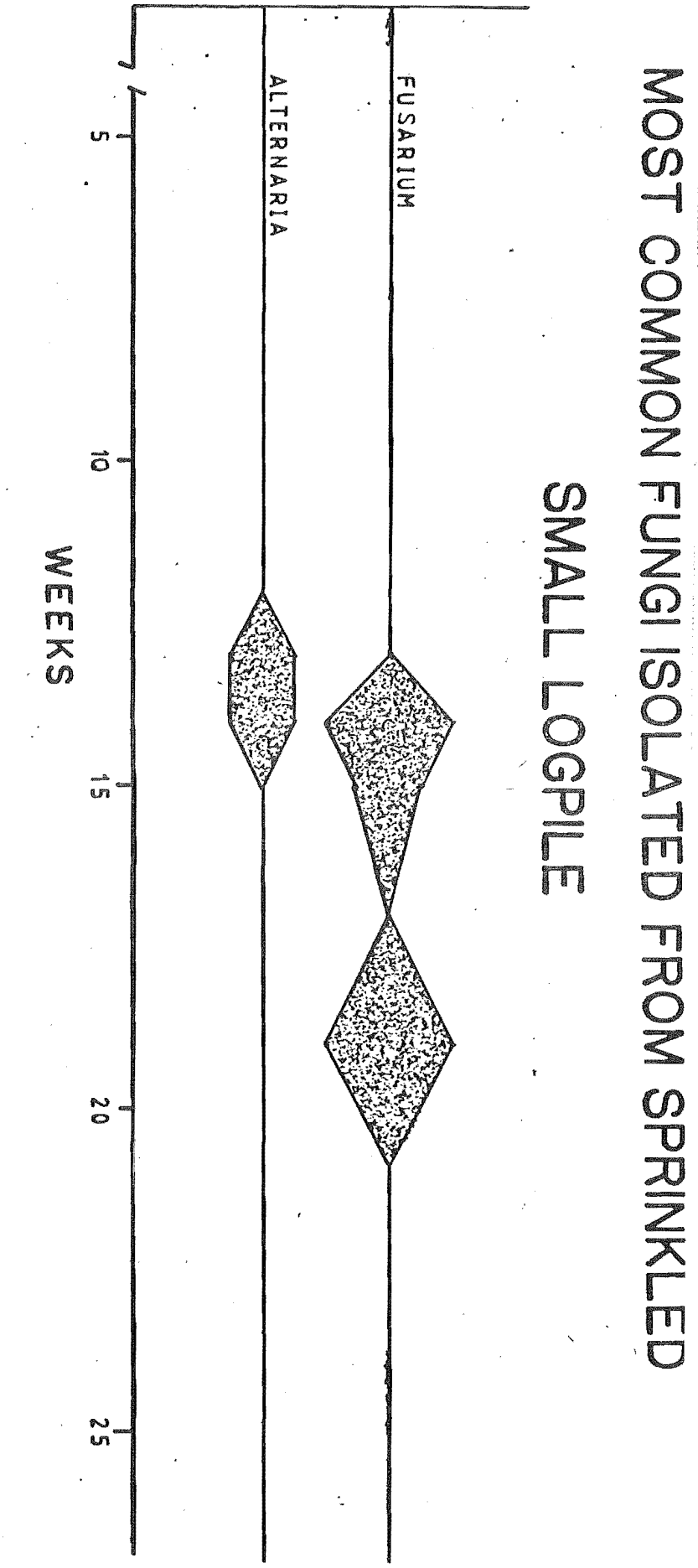
Figure 6:1 illustrates that no activity was observed until the tenth week. From this time isolations from the unsprinkled pile increased rapidly, reaching their highest level by the fifteenth week, but showing great variation over the whole sampling period. Isolations from the sprinkled pile appeared after thirteen weeks, peaked almost immediately and tailed off again at fifteen weeks, at which level they remained until the nineteenth week after which no further isolations were obtained.

The general pattern of colonisation in the sprinkled and unsprinkled piles is illustrated in Figures 6:2 and 6:3 where fluctuations are shown for the most common fungi isolated. The frequency of isolations of all fungi from both piles is given in Appendix F4.

In the unsprinkled pile the only fungus isolated for the first three weeks was *Alternaria*. A fungus capable of causing blue stain was therefore present right at the start. This was succeeded by *Fusarium* which remained dominant for the next three months. *Trichoderma* appeared briefly after

Figure 6:2




MOST COMMON FUNGI ISOLATED FROM SPRINKLED
SMALL LOGPILE
FUSARIUM
ALTERNARIA
5
10
15
20
25
WEEKS

# MOST COMMON FUNGI ISOLATED FROM UNSPRINKLED SMALL LOGPILE

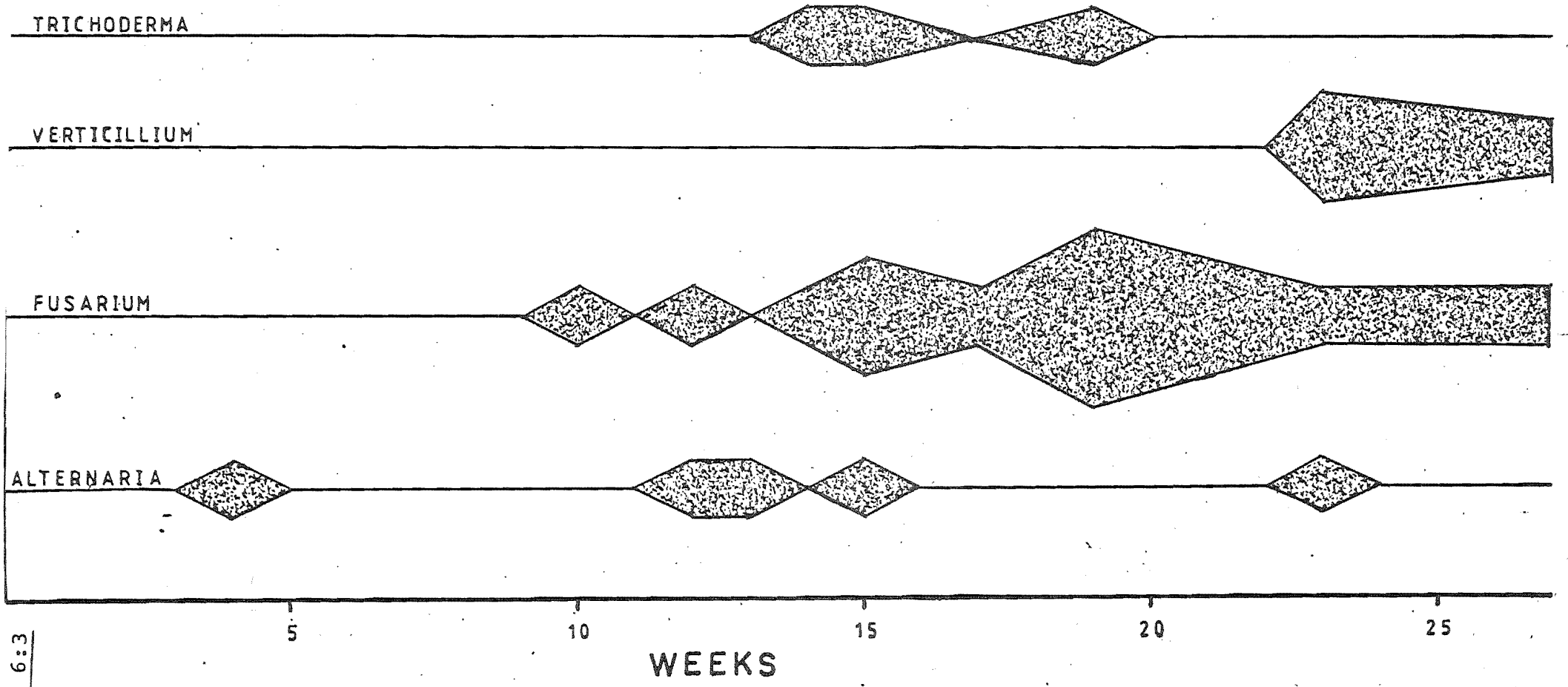



Figure 6:3

three months but was not isolated after five months. However after six months *Verticillium* took over as the dominant fungus with *Fusarium* declining in numbers. *Alternaria* appeared throughout spasmodically.

In the sprinkled pile both *Fusarium* and *Alternaria* appeared after three months but lasted only a short time. After five months no further fungi were isolated.

It is unlikely that these results, based on periodic isolations, give the complete picture: however they closely resemble those found by other workers (Butcher 1968; Bannerjee and Levy 1971). The pattern of blue stain to mould fungi is the same as that found by Butcher (1968) for the above ground portion of *Pinus radiata* stakes. The relative lack of diversity of organisms could be due to several factors. This part of the project was carried out in winter and this could account both for a slower buildup than that shown by Butcher and for the lack of diversity. However of equal or greater importance could be the isolation technique. In this study only the one isolation medium, 2.5 percent malt agar, was employed while Butcher also employed Abrams plus cellulose medium and Bannerjee and Levy (1970) used 2.5 percent malt agar alone or with Rose Bengal and streptomycin; malt agar with o phenyl phenol; Czapek Dox; Abram medium and Whatman cellulose medium.

In this study the pattern of colonisation in the unsprinkled logs had only reached the mould stage while the pattern in the sprinkled logs went no further than blue stain. Earlier isolations from the Balmoral logpile had shown that after three years of sprinkling the most common fungi being

isolated were mould fungi and after four years some soft rot and basidiomycete fungi were isolated. Therefore it could be postulated that in fact over a very extended time scale the full succession of fungi as outlined by Käärik (1974) would be encountered. A more definite appraisal of this time scale could be very important when trying to determine a safe storage period for logs under sprinklers. In fact the relationship of findings in this project to a definite storage time will be mentioned throughout in the hope of giving a clearer guideline as to the best storage time dependent of course on end use of the product.

6:3:2 Bacterial Isolations

All bacteria isolated from the logpile were examined by the range of tests set out in Appendix F5. Although not all isolates were examined by the full range of tests, most were. All samples taken were incubated aerobically and anaerobically, the isolations being made and anaerobic incubations as in Appendix F4.

Initially in this study it was hoped to use a simple determinative scheme based largely on presumptive methods as outlined by DeGroot and Johnson (1976). Their procedure was not to be followed exclusively but combined with the determinative keys employed when using the commercial 'Minitek' method of identification. Preliminary work had shown that a wide range of genera could be expected. It was also recognised that many would fall into the range 'coryneform' and 'coliform' groupings.

In an attempt to obtain the most information from the tests used to characterise the isolates, numerical

taxonomic methods were employed to achieve some measure of grouping. From these groups selected isolates were further characterised.

A two state coding method was employed (Sneath 1972) with all characters. Characters were computed in a n x t matrix and an estimation of resemblance was calculated using a simple matching coefficient

$$S_{sm} = \frac{a + d}{n}$$

Coefficients of similarity were then compared using an unweighted pair group cluster analysis and a dendogram constructed. Cophenetic values between all pairs of forms were calculated and the values obtained were compared with the similarity values on which the dendogram was based. The programme used was derived from 'plant cluster' held by F.R.I. Christchurch.

The groupings obtained in this study in no way constitute a taxonomic grouping. The limited number of characters used (30-32) are insufficient for a numeral taxonomy. Sneath (1972) suggests that a minimum of about sixty characters should be employed and if possible there should be 100-200. However Sneath and Sokal (1973) also admit that the requirement of sixty characters cannot be justified on either empirical or theoretical grounds. The correct number of characters is related to the problem of the congruence of classification based on sets of characters of the organism. Since congruence is always less than expected from random samples of characters, the numbers of characters used set a lower limit to the confidence levels of the similarity coefficients. Hence although the number of

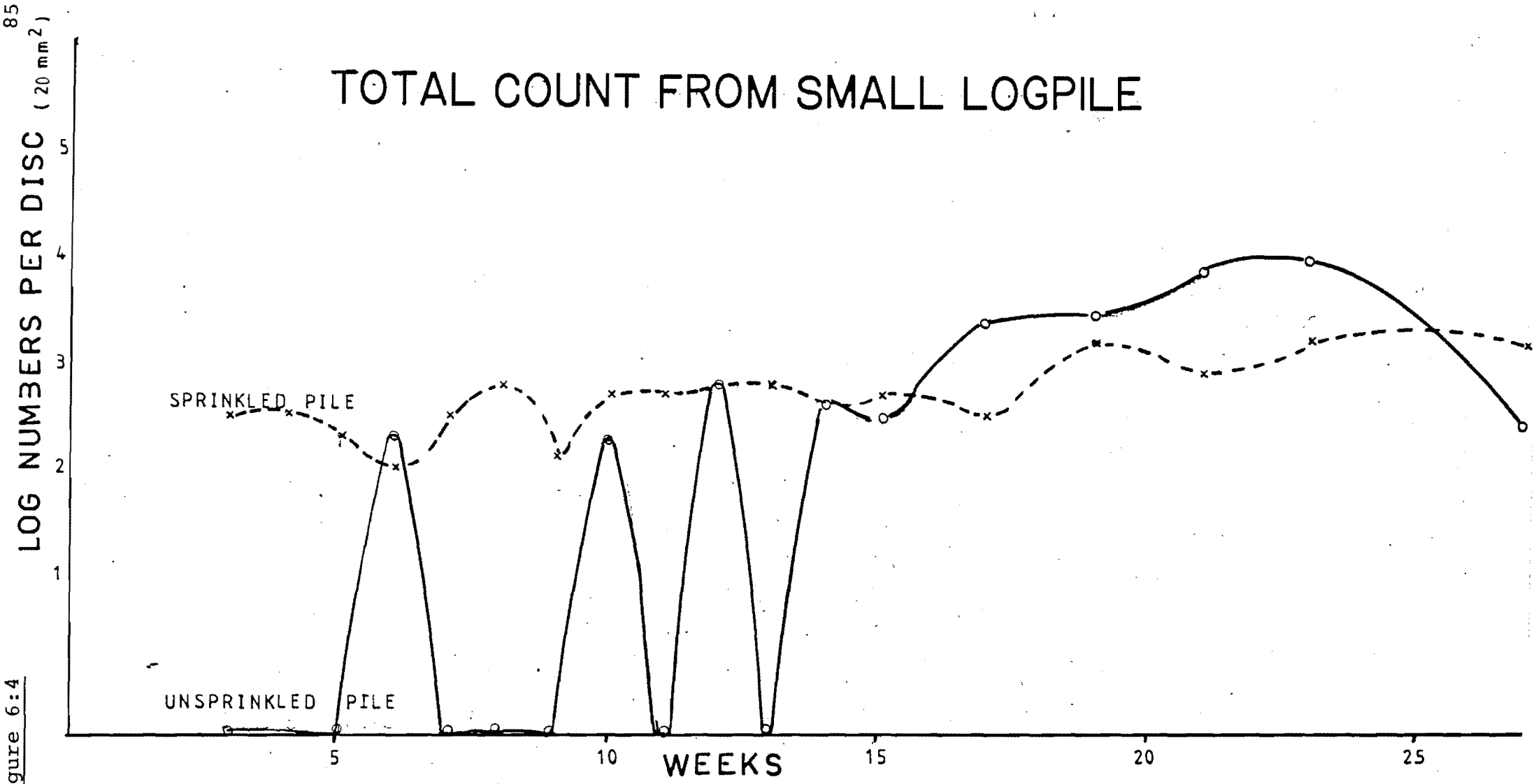

TOTAL COUNT FROM SMALL LOGPILE
LOG NUMBERS PER DISC ( 20 mm² )
1
2
3
4
5
SPRINKLED PILE
UNSPRINKLED PILE
5
10
15
20
25
WEEKS

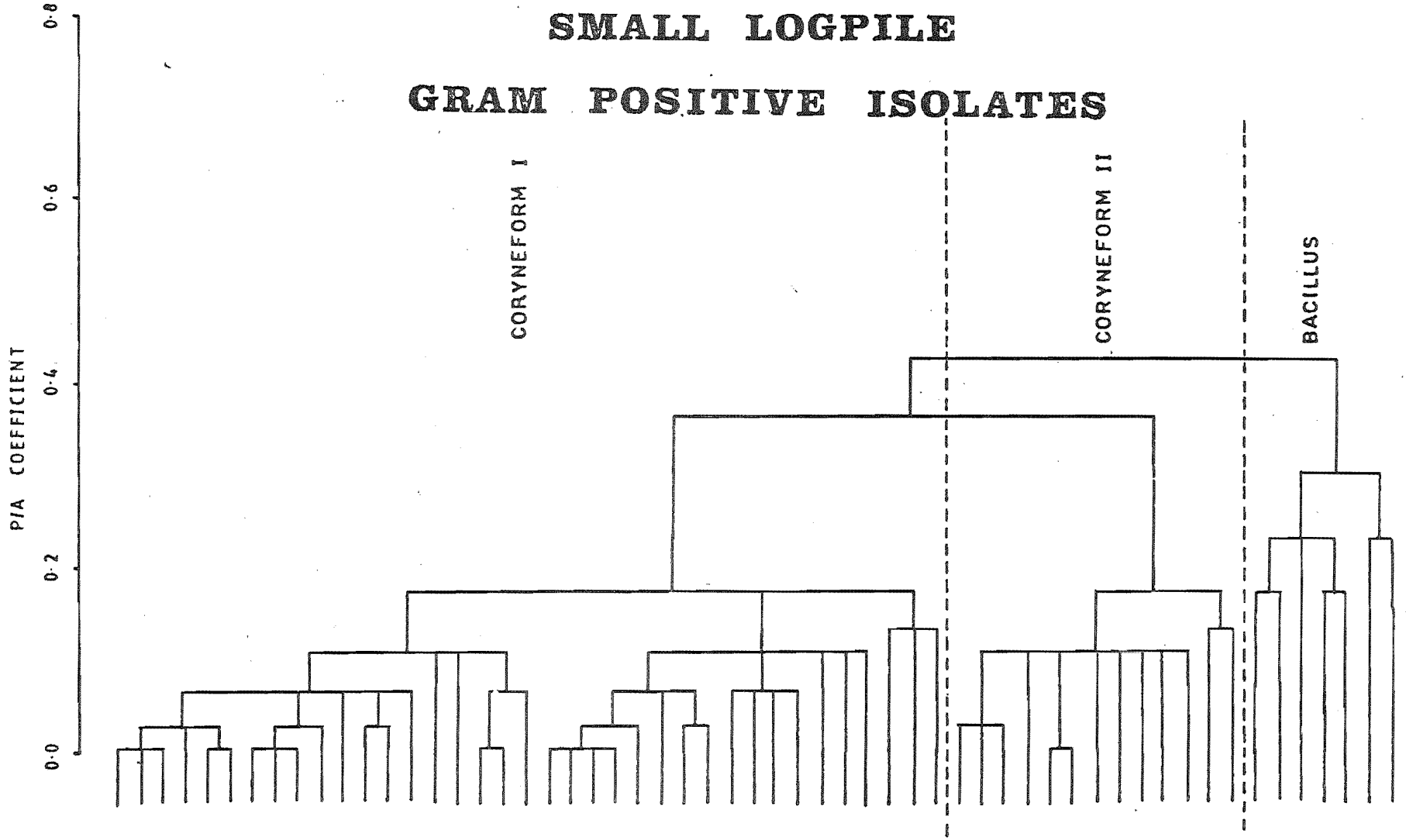
Figure 6:4

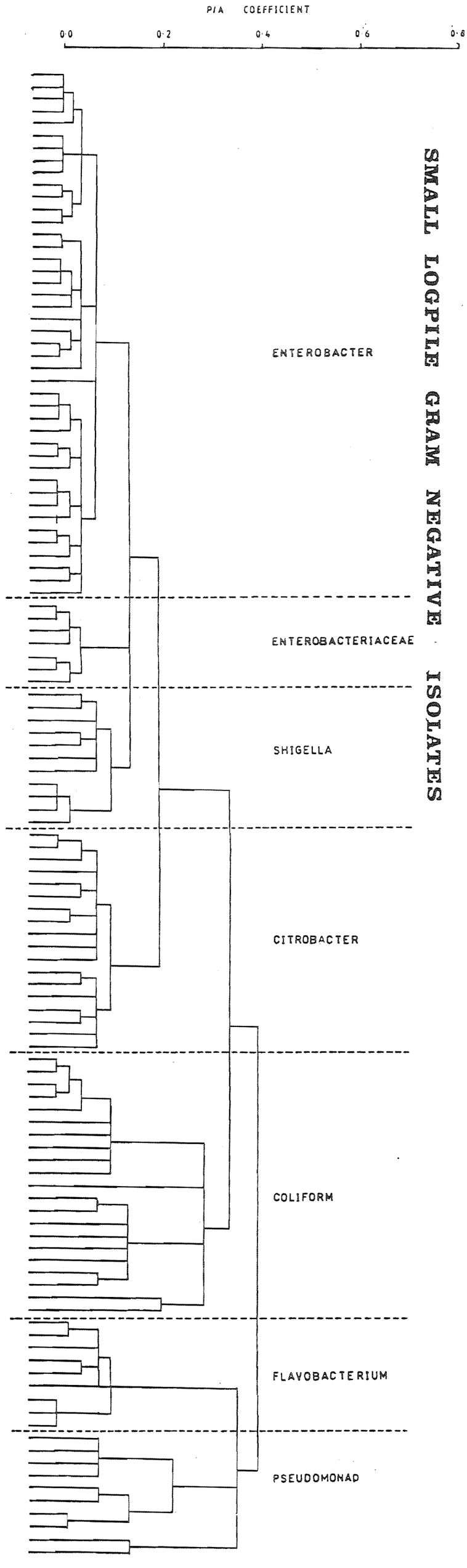
characters employed in this study could validly be used to generate taxonomic separation the confidence limits would render questionable the validity of the genera created.

Therefore, as stated, the groupings obtained were treated as such, merely convenient groupings. That the degree of similarity within most groupings was close only rendered this approach more useful.

The total counts obtained (Figure 6:4, Appendix F6) show how the two piles differed from each other. The sprinkled pile showed high numbers of bacteria after three weeks. This high population level was maintained virtually throughout the length of the study, increasing slightly after the seventeenth week of sprinkling. The number of isolates taken for characterisation also reflects this, with 130 out of 176 isolates being obtained from the sprinkled pile. The unsprinkled pile showed a marked degree of fluctuation in the population recorded for the first fourteen weeks of the study. After this time the population increased rapidly and maintained a level much higher than the sprinkled pile for most of the remaining sampling period.

The result from the small logpile therefore indicates that sprinkling does have some effect on the bacterial numbers in wood. The effect of sprinkling with water would appear to be an amelioration of conditions, perhaps to the extent of insulating the wood and therefore the bacteria to any rapid change in the external environment. This contention is supported by the marked fluctuations in the bacterial numbers in the unsprinkled pile during the early establishment phase.


SMALL LOGPILE
GRAM POSITIVE ISOLATES
CORYNEFORM I
CORYNEFORM II
BACILLUS
P/A COEFFICIENT
0·0
0·2
0·4
0·6
0·8


P/A COEFFICIENT
0·0
0·2
0·4
0·6
0·8
SMALL LOGPILE GRAM NEGATIVE ISOLATES
ENTEROBACTER
ENTEROBACTERIACEAE
SHIGELLA
CITROBACTER
COLIFORM
FLAVOBACTERIUM
PSEUDOMONAD

From the samples taken 176 isolates were isolated and tested to the characters outlined in Appendix F5. With the results a dendogram (Figure 6:5) was constructed using the similarity indices obtained from the similarity matrix. Ten groups were separated using all the listed characters and these are described below. Indentification of the aerobic bacteria was achieved with reference to the schema of DeGroot and Johnson (1976) and Berndt and Liese (1973). Further definition of the *Bacillus* grouping was aided by the description of Wolf and Barker (1968). Skerman (1967) was used for most of the other groupings while Edwards and Ewing (1972) was consulted for the enterobacteriaceae groupings.

Group I (*Citrobacter*) This homogenous group consisted of fourteen isolates. All isolates were gram negative, indole negative, Voges-Proskauer negative, $H_2S$ positive (12/14), catalase positive, oxidase negative, with nine out of fourteen being motile. None of the isolates could utilise cellulose but 13/14 could utilise pectin and hydrolyse starch.

Group II (*Enterobacter*) This group was the largest with 46 isolates. The group was not homogeneous but nevertheless did form a distinct grouping as Enterobacter. Isolates were all gram negative, indole negative, Voges-Proskauer negative (42/46), citrate positive, $H_2S$ negative, catalase positive (40/46), oxidase negative, with 37 out of 46 being motile. Most could utilise pectin (44/46) and starch (45/46) and fifteen isolates could utilise cellulose.

Group III (coliform) The reactions of this group of 25 isolates suggested that they might be in the genus

*Erwinia*. However without more definitive tests this could not be confirmed. The group itself was quite compact with only minor deviations. Isolates were all gram negative, fermentative, indole negative, Voges-Proskauer negative, nitrate positive, catalase positive, oxidase negative, with eighteen out of 25 being motile. Cellulose was utilised by 5/25 isolates, 17/25 could utilise pectin and 18/25 hydrolyse starch.

Group IV (*Flavobacterium*) This group of eight isolates consisted of yellow pigmented, gram negative rods, producing no acid in the Hugh and Leifson test: this identifies them as *Flavobacterium*. They were also catalase positive, 6/8 were motile, 5/8 utilised cellulose, 7/8 utilised pectin and hydrolysed starch.

Group V (*Shigella*) This group of eleven isolates was more variable. All isolates were gram negative, indole negative 8/11, Voges-Proskauer negative, $H_2S$ negative, urease negative, catalase positive and oxidase negative. This is sufficient to place them in the genus *Shigella* (as defined by Edwards and Ewing 1972) although most gave a positive reaction to citrate, 8/11, which is not quite in keeping with this genus. No isolates were motile, all reduced nitrate to nitrite, none could utilise cellulose but all could utilise pectin and hydrolyse starch.

Group VI (Enterobacteriaceae) This group of seven isolates was quite distinct. However the results from the tests make it difficult to place this group, other than in the family Enterobacteriaceae. Despite this the group was perhaps one of the most interesting, with all isolates being

able to utilise cellulose and pectin as well as hydrolyse starch. All but one of the isolates were motile, all were gram negative small rods, indole negative, Voges-Proskauer negative, $H_2S$ negative, catalase positive and oxidase positive.

Group VII (*Pseudomonads*) All eleven isolates in this group were gram negative rods with the ability to produce a fluoroscene compound, turning nutrient agar a brown colour. The isolates were all oxidative, catalase positive, nitrate negative, and could therefore be regarded as *Pseudomonads*. None showed motility, 3/11 could utilise cellulose, 9/11 pectin and all could hydrolyse starch.

Group VIII (coryneform I) All isolates in this group of 34 were gram positive short rods. All were catalase positive and oxidase negative and 32/34 reduced nitrate. Only 6/34 isolates could utilise cellulose, 28/34 could utilise pectin, 30/34 could hydrolyse starch, while 29/34 were motile.

This group was quite distinct from the next group which also exhibited characters of the coryneform group as defined by Skerman (1967).

Group IX (coryneform II) All isolates were gram positive long narrow rods. All were catalase positive and oxidase negative, nitrate negative, Voges-Proskauer negative and fermentative. Most isolates (12/13) hydrolysed starch and 9/13 utilised cellulose and pectin.

Group X (Aerobic spore forms) This group of seven isolates gave variable results to several tests: however all are gram positive and show endospore formation. Only 2/7 could utilise cellulose, 5/7 pectin, and 6/7 hydrolyse starch.

The characters related above allow the bacteria isolated to be fitted approximately into a classical classification scheme. However in this study it is perhaps more realistic to concentrate on those characters which give to the bacterium the best chance of survival or exploitation of the environment. To exploit wood, the bacteria must at some stage possess or develop the ability to break down some of the major components which go to make up wood. Although it could rely for a while on simple sugars and starches, these must become exhausted and if the bacterium is not to die out, it must start breaking down pectins, hemicelluloses, celluloses, etc. The ability to move through this environment might also be advantageous for it although Greaves (1971) did not find motility to be necessary for wood inhabiting bacteria.

Table 6:4 shows the reactions of the various groups to those characters which test the organism's ability to survive in a 'wood' environment. Most isolates could hydrolyse starch so gaining them a good foothold in wood while this compound was still available. Most could also utilise pectin. This is important as many structures in the wood cell are pectinaceous, e.g. the torus in pits. However, not many isolates had the ability to utilise cellulose. This test was not an exhaustive one as the only measurement of cellulose utilisation was the ability to grow on solid media containing cellulose as the sole carbon source.

Table 6:4

Percentage Positive results of Selected Tests on Isolates from the Small Logpile

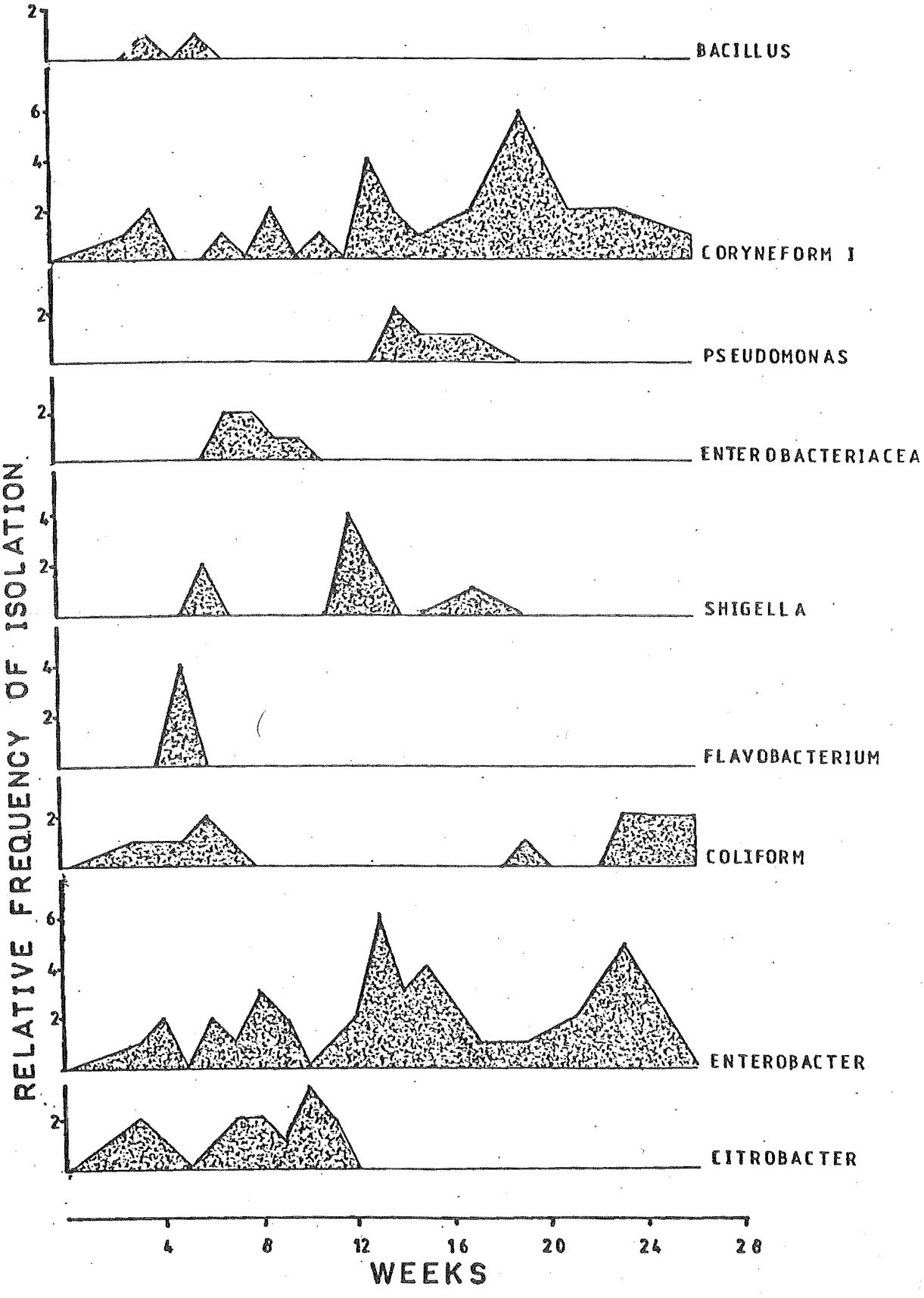
| Tests | Groups | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| Gram | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 100 |
| Motility | 64 | 80 | 72 | 75 | 100 | 86 | 100 | 85 | 62 | 14 |
| Cellulose | 0 | 33 | 28 | 63 | 0 | 100 | 27 | 18 | 70 | 71 |
| Pectin | 93 | 96 | 68 | 87 | 100 | 100 | 82 | 82 | 70 | 71 |
| Starch | 93 | 98 | 72 | 87 | 100 | 100 | 100 | 88 | 92 | 86 |
| Isolate number | 14 | 46 | 25 | 8 | 11 | 7 | 11 | 34 | 13 | 7 |

The ability of most isolates to degrade pectin suggests that pectinaceous materials in the wood could be broken down e.g. around the pit. Numerous workers (e.g. Schmidt 1978; Boutelje and Göransson 1971) have isolated bacteria capable of degrading pectin and work outlined in Chapter III indicated that the pits have been broken down. The group of isolates (group VI) which were difficult to identify except as belonging to the Enterobacteriaceae showed the greatest ability to degrade cellulose; all seven isolates could break down cellulose powder.

Figure 6:5 shows how the isolates appeared in successive waves. Three different stages in the succession can be recognised. Most of the groups identified were isolated in the first stage; *Pseudomonas* being the only group not isolated until the second stage. *Bacillus*, Enterobacteriaceae, *Flavobacterium* and *Citrobacter* isolates

Figure 6:6

# BACTERIAL ISOLATIONS FROM SMALL LOGPILE



are all only isolated in the first stage of the succession. Only the enterobacter and coryneform I groups are isolated in all three stages. These two groups are also the largest isolated. Enterobacter shows a cyclic pattern of isolation with a peak in each stage of the succession. Coryneform I, although isolated in all stages are most numerous in the second stage of the succession. The group identified as from the enterobacteriaceae were isolated only in the first stage of the succession despite their displaying an ability to break down cellulose. Enterobacter which were found in all stages of the succession had only 33 percent of the isolates capable of breaking down cellulose and coryneform only had 18 percent capable.

However it is evident that although a succession of bacteria can be demonstrated there is no succession in enzymetic ability. Groups of isolates which could all break down cellulose appear only in the first twelve weeks of sprinkling whereas a group which has only a few isolates capable of degrading cellulose and only 68 percent capable of degrading pectin (coliform) is found after 22 weeks of sprinkling as well as at the beginning. Clearly the succession of micro-organisms found in the sprinkled wood depends on more than just the ability to break down simple carbohydrates followed by more complex carbon sources.

# CHAPTER VII

## PRESERVATION OF WOOD BY SPRINKLING

Moisture is probably the most important factor that governs the activity of wood decay fungi, with brown rot fungi in general having a lower moisture requirement than white rot fungi (Highley 1978). But obviously fungi have other requirements necessary for successful invasion and degradation of wood: Snell (1929) found for instance that an amount of air, in excess of 20 percent of the wood volume was necessary. This figure is also mentioned by Hansbrough (1953) who goes on to suggest that this is the reason why water-logging of wood is so successful in restricting fungal growth.

Scheffer (1969) claims that it is common knowledge that spraying affords protection to logs by restricting the diffusion of oxygen. He suggests that there is little evidence to suggest that green logs take up much of the water but that the action of spraying is to maintain the initially high moisture content. The growth of miscellaneous micro-organisms on the ends of logs may also assist by precluding the passage of oxygen into the log by way of the ends. Moltesen (1977) also agrees with the supposition that the film of micro-organisms that develops on cut ends restricts the entry of oxygen.

Lack of nutrients has also been suggested as a reason for the lack of fungal invasion. Paserin (1970) found that living cells could be found in water stored wood after five

months and suggested that some of the cells' reserves were used in respiration. Some work by King, Oxley and Long (1975) and Oxley, King and Long (1976) has shown that invading fungi need high levels of some nutrients, especially nitrogen, for the breakdown of wood. They suggest that soft rot fungi particularly require high levels of nitrogen whereas basisiomycetes are less affected. Drying of wood was shown to affect the distribution of nutrients, there being a concentration on the outside as drying proceeded. From this it seems likely that continual wetting would slow this concentration and even remove some of the more water soluble material. Levy *et al.* (1974) contend that high nitrogen levels are required for successful basidiomycete growth, and that much of this comes from nitrogen fixation by bacteria within the wood. Work by Butcher (1978) shows that a low ratio of nitrogen to carbon favours soft rot decay. He also surveys other published work on soft rot indicating that soft rot organisms are most active at high moisture contents; for instance one study showed a moisture content of 107-144 percent to be optimal in pine. Soft rot fungi also tolerate poor aeration and there is some indication that carbon dioxide may be stimulatory.

Moisture contents in excess of 150 percent did not prove inhibitory to a selection of wood decay fungi tested by Ammer (1964). The level reached before there was a reduction in growth was over 170 percent in most cases, the only exception noted being *Coniophora puteana* where there was a gradual decline after a moisture content of 60 percent was

reached. Highley (1978) also found that high initial moisture content did not stop the establishment of decay in sapwood of softwoods or hardwoods.

Other factors reported to be operating in the protection of wood under water are temperature and interactions between fungi and bacteria. Scheffer (1969) suggests that the cooling effect of spraying may help in protecting wood and Hansbrough (1953) states that temperature has a strong influence on fungus activity with little or no growth at temperatures below 4.5$^{0}$C or above 49$^{0}$C. Because of this Hansbrough contends that water protection is necessary only during the summer months. In reviewing the literature Smith (1975) found reports of both inhibition and stimulation of wood destroying fungi by bacteria when investigating these interactions in wood.

It is clear that the reasons why water protects wood from decay are imperfectly understood, so an attempt was made to determine the mechanisms of protection in the Balmoral logpile. The level of aeration, effects of temperature and possible interactions between fungi and bacteria were studied. Since the main thrust of the project was to determine the level of degradation with a view to predicting a safe storage period for *Pinus radiata*, these tests of the mechanisms of protection were relatively superficial. The aim was to provide some insight into the problem with the hope that the results would help in further, and more detailed studies by other researchers.

## 7:1 GASES IN WATER SOAKED WOOD

Studies on how long wood destroying fungi can endure immersion in water by Schmitz and Kaufert (1938) showed that for three fungi, immersion for 268 days had no deleterious effect. During this time no measure of any decomposition of the wood was made. However Walsh (1971) found that the efficiency of carbon utilisation by wood rot fungi fell at low oxygen tensions. Lagerberg, Lundberg and Melin (1927) found when investigating stain fungi that a supply of oxygen was necessary for the growth of the fungi at high wood moisture contents.

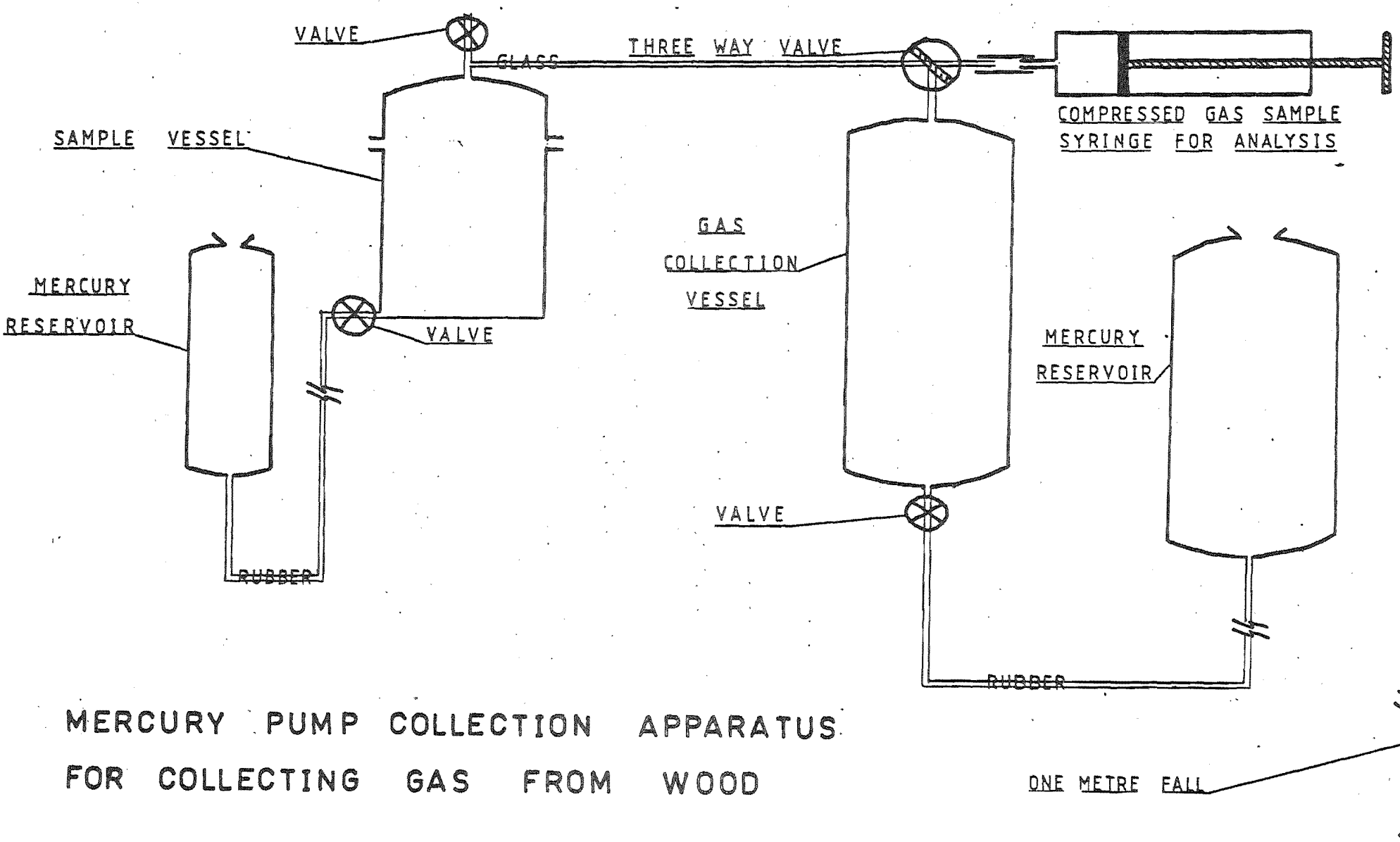
They did not determine the amount of oxygen needed but did state that a moisture content of 125 percent was near the upper limit for most of the fungi they investigated in spruce and pine. Scheffer and Livingston (1937) investigated the growth of *Polystictus versicolor* at nine different partial pressures. They found that the minimum oxygen pressure for mycelial growth was between 1.5 and 10 mm.

Measurement of the composition of air in sugar maple by Thacker and Good (1952) revealed that the oxygen level was lower in the centre of the tree and lower in decayed wood. They found the oxygen content in all trees tested was always below atmospheric, the total range found being 0.8 percent to 19.2 percent while carbon dioxide was 1.6 percent to 16.9 percent. However in sound wood there was comparatively little variation, the range for oxygen being 12.4 percent to 17.7 percent and 4.8 percent to 1.9 percent for carbon dioxide. MacDougal and Working (1933)

found similar levels in live *Pinus radiata*; 15-20 percent for oxygen and 1.2 percent to 4.2 percent for carbon dioxide. Jensen (1967) sampling from red oak trees obtained oxygen levels of 5.5 percent to 7.5 percent and carbon dioxide levels of 13.5 percent to 16.5 percent. He also noted that the longer the collection time the higher the carbon dioxide level recovered up to a level of 50 percent, a difference he associated with leaks over long time periods. However different levels were recorded by Carrodus and Triffet (1975) from *Acacia mearnsii*. They found carbon dioxide levels of greater than 90 percent in outer sap decreasing to 40-65 percent from inner sap and 40 percent from outer heart. Oxygen levels found were very low accounting for no more than 2.5 percent in any sample. Buston, Moss and Tyrrell (1966) have shown that some carbon dioxide is stimulating to fungal growth as well as the necessity of oxygen. A measure of the gases available in the Balmoral wood after four years' sprinkling was therefore determined.

### 7:1:1 Gas from Balmoral water stored Wood

While the logs were completely wet, oversize samples approximately 120 x 250 mm were taken from the logs. This was achieved by sawing off a disc 250 mm thick and rapidly splitting out the required sample. These samples were immediately immersed in molten wax to seal them, several dippings being made to ensure a thick, complete seal. The waxed samples were then immersed in water and taken back to the laboratory. Here they were trimmed under water to a cylinder with length 100 mm and diameter 75 mm. The number




MERCURY PUMP COLLECTION APPARATUS FOR COLLECTING GAS FROM WOOD

Figure 7:1

of samples taken was such that not all could be processed in one day. Therefore after final sizing to 70 x 100 mm under water, all cylinders were immediately recoated with wax and stored in water at $5^0$C until gas was extracted ; with all gas samples being taken within one week of initial sampling from the logpile. Immediately before gas was extracted the wax was cut off each end of the cylinder and the end fibres cut cleanly with a chisel; all this accomplished under water. The cylinder was then placed in the sample vessel (Figure 7:1), lid sealed and all air excluded by mercury. The mercury reservoir was then lowered to create a partial vacuum, the total differential being 800 mm. The gas sample gathered was drawn off by syringe and stored in a vacuum tube (BBL Vacutainer) until analysed on a Varian series 1520 gas chromatograph using either a poropak Q or molecular sieve packed column.

Taking the percentage saturation as 92 percent in the outer sapwood and 40 percent in the heartwood (see Chapter V) the theoretical volume of gas is 25 ml within the sapwood samples and 170 ml in the heartwood (calculations Appendix G1). Extraction of 10 ml of gas for analysis from sapwood took approximately six hours while 10 ml of gas from heartwood was obtained within 30 minutes. Hence about 40 percent of the available gas was extracted from the sapwood and 5 percent from the heartwood.

The results of the analysis of gas samples are shown in Table 7:1, statistical analysis in Appendix G2 and calibration curves in Appendix G3. The general level of oxygen found in both heartwood and sapwood compares very well

with the levels found by Thacker and Good (1952) in sound wood. In this study a higher level was found in the heartwood (15.3 percent) than in the sapwood (11.1 percent) while the level of carbon dioxide was higher in the sapwood. This is to be expected as earlier work (Chapter VI) has shown that there was more bacterial action in the sapwood. Of more importance is that at these levels of oxygen, fungi could be expected to grow. Thacker and Good (1952) also determined the concentration of oxygen needed for growth of fungi isolated from sugar maple and found levels less than 10 percent adequate. They noted that carbon dioxide concentrations up to 10 percent were not inhibitory, while at concentrations between 15 to 20 percent inhibition was apparent but growth still occurred. The results of McDougal and Working (1933) from *Pinus radiata* also compare very closely to the results of this study.

The results show that whereas low level of oxygen was recorded, a high level of carbon dioxide was found. This was particularly evident in the sapwood samples and may relate to the level of microbial activity that was found. It also supports the observation that all bacteria isolated were either aerobic or facultative (see Chapter VI) as the level of oxygen found is sufficient for the growth of aerobic bacteria. Results obtained indicate that the level of oxygen found in the gases extracted from the sprinkled wood was enough to support fungal growth. This study was concerned with the level of oxygen, carbondioxide and nitrogen after four years' sprinkling. No suggestion is made that oxygen levels were the same throughout the four years'

Table 7:1

Analysis of Gas samples taken from sprinkled Balmoral Logpile

| | Oxygen % | Carbon Dioxide % | Nitrogen % |
|---|---|---|---|
| Heartwood | 18.2 | 0.0 | 64 |
| | 25.6 | 0.8 | 74 |
| | 18.0 | 2.7 | 67 |
| | 8.8 | 3.5 | 60 |
| | 16.4 | 2.7 | 67 |
| | 9.4 | 7.2 | 50 |
| | 10.8 | 11.0 | 56 |
| Sapwood | 18.6 | 1.0 | 63 |
| | 3.6 | 0.0 | 7 |
| | 14.2 | 4.9 | 61 |
| | 24.6 | 4.0 | 72 |
| | 17.0 | 7.7 | 50 |
| | 6.8 | 18.6 | 63 |
| | 5.4 | 14.4 | 47 |
| | 4.6 | 7.9 | 72 |
| | 5.6 | 8.2 | 63 |
| | 16.0 | 7.9 | 71 |
| | 6.0 | 14.0 | 77 |
| Average heartwood | 15.3 | 3.9 | 62.6 |
| Average sapwood | 11.1 | 8.1 | 58.7 |

sprinkling. The composition of the gas within the wood could have been quite different earlier and probably was. The composition of the gas after four years' sprinkling only indicates that at that time oxygen and carbon dioxide levels were suitable for fungal invasion if no other factors were inhibitory.

## 7:2 EFFECTS OF TEMPERATURE ON WOOD ROTTING FUNGI

Work by a number of authors has shown that stain fungi can tolerate low temperatures. Lagerberg, Lundberg and Melin (1927) found that growth of most of the fungi they studied could be detected at $3^0$-$4^0$C. Lindgren (1942) and Henningsson and Lundström (1974) have also shown growth at $5^0$C for a large number of stain fungi. They have also indicated that $25^0$C is the optimum temperature for most. Pechmann (1965) found that for some stain fungi growth occurred at temperatures approaching $0^0$C and that short periods below $0^0$C did not adversely affect stain fungi but appeared to increase their growing capacity when temperatures lifted.

Humphrey and Siggers (1933) working with a large number of wood destroying fungi found that most recorded some growth at $12^0$C, the lowest temperature tested.

### 7:2:1 Effect of temperature and bacteria on rot fungi

Fresh *Pinus radiata* from a compartment in Balmoral of similar age to that in the sprinkled logpile was obtained and 96 blocks 20 x 20 x 20 mm were cut and weighed. These were divided into two groups of 48 and autoclaved in 2-litre

flasks containing nutrient broth. One flask was inoculated with a mixed culture of motile and nonmotile gram negative rods cultivated from *Pinus radiata* and incubated at 25°C for one week, the other flask remained sterile. After incubation the blocks were aseptically placed on trays of soil inoculated with active cultures of either *Coniophora puteana* or *Polystictus versicolor*. This test was the same as the soil jar test (ASTM D1413) except that foil trays were used, each tray capable of holding 16 blocks. Thus six trays were prepared, two of which were then incubated at each of three temperatures, 5°C, 15°C and 25°C. This is summarised below:

Table 7:2

Effects of selected Temperatures on two Wood Rotting Fungi

| | *Coniophora puteana* | | *Polystictus versicolor* | |
|---|---|---|---|---|
| | bacteria | no bacteria | bacteria | no bacteria |
| 5°C | 8 blocks | 8 blocks | 8 blocks | 8 blocks |
| 15°C | 8 blocks | 8 blocks | 8 blocks | 8 blocks |
| 25°C | 8 blocks | 8 blocks | 8 blocks | 8 blocks |

All trays were incubated for twenty weeks after which the blocks were removed, dried and weight changes determined.

Analysis of the results (Table 7:3 and Appendix G4) highlights the significant difference in weight loss between the three temperatures. However even at the lowest temperature of 5°C weight losses of over half those recorded for 15°C are shown. This relates well to earlier work

outlined above. There was also a significant difference between the amount of decay caused by the two rot fungi chosen. Presence or absence of bacteria in the wood did not significantly affect weight losses: stimulation or inhibition of the fungi by the bacteria was therefore not demonstrated.

Table 7:3

Summary of weight losses in *Pinus radiata* caused by wood rotting fungi at different temperatures

(weight loss $kg/m^3$)

| | *Coniophora puteana* | | *Polystictus versicolor* | |
|---|---|---|---|---|
| | bacteria | no bacteria | bacteria | no bacteria |
| 25°C | 35.0 (a) | 32.5 (b) | 37.5 (g) | 32.5 (h) |
| 15°C | 32.5 (c) | 32.5 (d) | 23.75 (i) | 21.25 (j) |
| 5°C | 20.0 (e) | 25.0 (f) | 20.0 (k) | 15.0 (l) |

See Appendix F for Test for equal sample variances

To relate these temperatures to those occurring in the Balmoral logpile, temperatures within the logs were recorded over the winter and spring months. No recordings were made in summer as it was assumed that temperatures would be high enough for some growth at least. Thermocouple probes were embedded in twenty-four logs, twelve to a depth of 50 mm and twelve to a depth of 150 mm; twelve logs were near the top of the row and twelve were near the bottom. The results are summarised in Table 7:4.

Table 7:4

Temperature within logs under sprinklers at Balmoral

(means of six readings)

| | Date | | | | | |
|---|---|---|---|---|---|---|
| | 25-6 | 2-7 | 9-7 | 16-7 | 22-9 | 25-11 |
| Bottom logs | | | | | | |
| probe depth 50 mm | 7.5 | 9.5 | 6.0 | 3.75 | 9.75 | 8.75 |
| probe depth 150 mm | 7.75 | 9.5 | 5.0 | 3.50 | 9.5 | 9.5 |
| Top logs | | | | | | |
| probe depth 50 mm | 9.5 | 9.0 | 5.0 | 4.0 | 9.75 | 9.25 |
| probe depth 150 mm | 7.75 | 8.75 | 5.5 | 3.5 | 9.75 | 10.25 |
| Maximum air temperature | 7.5 | 17.0 | 12.0 | 7.0 | 15.0 | 15.0 |
| Minimum air temperature | 2.7 | 4.5 | 3.3 | -1.6 | 5.0 | 5.8 |

Despite air temperatures which varied from -1.6$^{0}$C to 17$^{0}$C the temperatures within the logs were relatively stable; the lowest reading recorded was 2.7$^{0}$C and most were above 5$^{0}$C at all times. These temperatures are not low enough to stop completely the activity of wood decay fungi.

The indication is therefore that for the wood stored under sprinklers at Balmoral neither the oxygen level nor the temperature was too low to inhibit the growth of wood decay fungi. The reason for the successful exclusion of even stain fungi for up to three years has not been determined.

With this in mind it was decided to attempt to determine whether bacteria inhibited the development of wood

decay fungi. An experiment was conducted where the wood was maintained in a moist condition and bacteria and fungi were added to the wood in various combinations.

## 7:3 INTERACTION BETWEEN FUNGI AND BACTERIA IN WOOD

According to Lapetite (1970) and Greaves (1970) the presence of bacteria in wood can severely inhibit subesequent attack by wood decay fungi. Two reasons have been suggested; direct competition between bacteria and decay fungi for nutrients, and secretion of substances by the bacteria which either inhibit growth of the fungi or inhibit cellulase production (Greaves (1970). However in some cases evidence of stimulation of fungal decay by bacteria was found, possibly by an effect on enzyme feedback-inhibition systems or by removal of toxic extractives. This evidence of both inhibitory and stimulatory effects of bacteria on wood decay fungi was also found by Aufsess (1967).

### 7:3:1 Fungi and Bacteria in Wet Wood

As outlined earlier a method was sought whereby the interaction between bacteria and fungi could be tested while maintaining the wood in a continually wet condition somewhat similar to sprinkled wood. The perfusion technique of Morton and Eggins (1976) (see also Eggins, Malik and Sharp 1968) with some modification proved satisfactory. The apparatus used is illustrated in Figure 7:2. Blocks of fresh end matched sapwood of *Pinus radiata* from Balmoral, 20 x 20 x 10 mm in size were randomly subjected to one of five treatments. The treatments were:

1) water soaked wood inoculated with *Coniophora puteana*
2) water soaked wood inoculated with mixed bacteria
3) water soaked wood inoculated with bacteria plus *Coniophora puteana*
4) water soaked wood inoculated with bacteria plus *Coniophora puteana* plus slime
5) water soaked wood inoculated with bacteria plus slime.

All blocks were autoclaved in nutrient broth before any subsequent treatment. Blocks with bacteria were incubated for one week at 25$^0$C in a mixed culture of motile and nonmotile gram negative rods isolated from the mini logpile (see Chapter VI). *Coniophora puteana* was grown on 70 x 10 x 2 mm *Pinus radiata* feeder strips on 2 percent malt agar until a good mycelial mat covered the strip. The fungus was introduced to the blocks by attaching the feeder strip aseptically to the blocks in the perfusion jar (see Figure 7:2). The slime was not identified except that initial culturing showed both slime producing bacteria and yeasts. This slime was collected from the cut ends of recently introduced wood under the sprinklers and spread over the appropriate blocks.

All pieces of the perfusion apparatus were sterilised before the experiment commenced and the water used was sterile distilled water. The perfusion units once set up were incubated at 25$^0$C for twenty weeks. The blocks were then redried, weighed and the weight losses per 400 mm$^2$ calculated.

The treatment which showed the greatest weight loss (Table 7:5) was *Coniophora puteana* alone. The bacteria alone

did not cause any significant weight loss. Bacteria inhibited the growth of *Coniophora puteana* as although mycelial strands could be found over the surface of the blocks their development was weak and little weight loss recorded. The addition of slime did not significantly increase the inhibitory action of the bacteria.

Table 7:5

Weight changes in wet wood inoculated with bacteria and *Coniophora puteana*

| Treatment | Weight loss (g/4000mm$^2$) | | | |
|---|---|---|---|---|
| A Fungus only | 0.1796 | 0.1896 | 0.1768 | 0.2268 |
| B Bacteria only | $4.07 \times 10^{-3}$ | $7.04 \times 10^{-3}$ | 0.0117 | $8.35 \times 10^{-3}$ |
| C Bacteria and Fungus | $2.00 \times 10^{-3}$ | $7.87 \times 10^{-3}$ | $5.89 \times 10^{-3}$ | |
| D Bacteria and Fungus and Slime | 0.013 | 0.011 | $9.89 \times 10^{-3}$ | 0.011 |
| E Bacteria and Slime | $8.25 \times 10^{-3}$ | $9.44 \times 10^{-3}$ | 0.011 | 0.011 |

See Appendix F4 for ANOVA and the test for Equal Sample Variances.

It can be concluded that the major reason for the failure of *Coniophora puteana* to rot wood in this experiment was the presence of bacteria. Water saturation alone was not sufficient to inhibit the growth and development of *C. puteana* in radiata sapwood. The mechanism of inhibition was not investigated in detail but inhibition by the excretion of a toxic substance seemed more likely than

competition for nutrients. *Coniophora puteana* was grown on a feeder stick which was included in the perfusion vessel. Visual checking throughout the twenty weeks' incubation found that when bacteria were present the profuse growth of *C. puteana* dwindled to a sparse network of mycelium within three weeks of the start of incubation. Mere competition for nutrients would be unlikely to cause this.

## 7:4 SUMMARY OF RESULTS

From these experiments it appears that prevention of fungal establishment by bacteria is the most likely mechanism preventing decay of wet wood. The levels of oxygen and carbon dioxide in the logs were within the limits tolerated by both stain and rot fungi. The temperature over the winter months was not so low as to preclude growth of fungi. This temperature presumably slowed the growth of fungi but some degrade could be expected even at $5^{0}C$ if all other conditions were not inhibitory. However it was found that bacteria do seriously inhibit the growth of a known wood decay fungus under laboratory conditions simulating those of water stored wood.

A valid criticism of these experiments is that only one aspect was considered at any one time. The exception was when temperature and bacteria were looked at together, in which case the effect of temperature was significant at the one percent level, but there was a less significant effect (5 percent significance) due to bacteria.

To establish properly the mechanism at work an experiment would have to be set up that allowed all aspects equal chance of operation while still being able to monitor the effects of each individual contribution. This series falls very short of this goal and can only be used to point to the possible mechanisms at work and the weighting each should have in a more comprehensive experiment. The experiment employing the perfusion apparatus has a maximum interaction of only three factors. The other experiments attempt to change one factor while controlling all others. However to do this adequately all influences on the establishment and advance of wood rotting fungi have to be tabulated. Conditions similar to those operating within a sprinkled logpile have to be reproduced. This series of experiments investigated a few conditions which other workers reported to be important. The results from these experiments have helped to establish which condition is the most likely for protecting wood under sprinklers rather than explain the interactions between the various factors.

# DISCUSSION AND CONCLUSIONS

# CHAPTER VIII

## DISCUSSION AND CONCLUSIONS

The purpose of this project was to ascertain the effects of storage of *Pinus radiata* D Don. under water sprinklers and predict this method's usefulness for long term log storage. This required examination of aspects of the biology and structural integrity of the wood.

Prediction of the maximum time of storage for *Pinus radiata* necessitated examining a wide range of factors; in prticular the microbial activity within the wood, the microscopic changes that occurred, the strength characters and the commercial aspects of treatability and transportability. These alone cover a broad field but by no means exhaust all the possible changes which might have occurred. Rather they were chosen in an attempt to describe the condition of the wood after three and four years' sprinkling, compared to freshly felled wood.

This has led inevitably to some frustrations where interesting discoveries have been made which call for a deeper examination. These have been pointed out in the hope that further work in this field will concentrate on these aspects.

Typical successions of fungi colonising wood have been described by various workers (Käärik 1974; Butcher 1968; Bannerjee and Levy 1970). Isolations from the Balmoral logpile after three and four years' sprinkling show that almost all isolates identified would have been placed in the

early stages of these successions. Only after four years' sprinkling were there any signs of rot organisms. The suggestion is made that the organisms isolated at Balmoral represent the start of a succession similar to those worked out by Butcher (1968) and Bannerjee and Levy (1970) for above ground wood but that the water spray has slowed the succession. This could not be verified in the present study because of the disposal of the logpile after four years' sprinkling. However if the appearance of a few rot organisms indicates a real change in the succession then it could be argued that the logpile has reached the maximum time limit for storage.

Bacterial isolations, particularly from the small scale logpile, also showed a successional change. The most important point to emerge from this study however was that the successional changes found did not appear to be related to the pectinolytic and cellulolytic activity of the isolates. The two groups of bacteria which were isolated most frequently and which therefore appeared the most successful inhabitants of the wood, (Enterobacter and coryneform bacteria) showed low cellulolytic ability when compared with another group of bacteria (identified as belonging to Enterobacteriaceae) which appeared briefly midway in the succession. Clearly the physiological characters chosen for study were not the most important for predicting the success of bacteria in exploiting sprinkled wood.

Microscopic examination of the wood showed that some damage to the wood structure had occurred after three years' sprinkling. The damage found was largely the loss of pit structures, namely margo and torus, rather than cell wall

damage. Damage to secondary cell wall thickening was only found in some cases after four years. This loss of some cell structures before three years' sprinkling may be related to the finding of bacteria with pectinolytic ability midway through the sampling period. Groups of bacteria were isolated which showed greater than 90 percent pectinolytic activity within twelve weeks of start of sprinkling.

The finding of a different winding angle in the $S_2$ fibrils of cellulose in some cell walls suggests a mechanism of disturbance to the interfibrillar matrix. Composition of secondary thickening is discussed by Kollman and Cote (1968) and Preston (1974) who show the matrix substances to include hemicelluloses, sugars and lignins with the 'hemicellulose' chains often lying parallel to the microfibril length. An apparent alteration in the winding angle of the cellulose microfibrils could well be caused by some loss to the matrix substances. Drying during S.E.M. preparation, although by critical point drying, could have set up some tension which with a lessened matrix complement caused a slight collapse in the spiral winding and hence an increase in winding angle from the expected $14^0$ to nearer $15^0$ to $20^0$. However this should give a drop in the modulus of elasticity, not an increase as found. The results of static bending also suggest a loss of matrix substance. A comparison of sprinkled and fresh wood showed that modulus of rupture was significantly higher, modulus of elasticity was slightly higher and work to maximum load showed no significant difference in four year sprinkled wood. Modulus of rupture measures the stress in the extreme fibres of the specimen

at the point of failure (Lavers 1969) while modulus of elasticity expresses the relationship between stress and strain. Thus the ability of the microfibrils to change orientation could affect these measures. It is suggested that during static bending the microfibrils change orientation to a degree due to the applied bending stress allowed by the loss of interfibrillar matrix substances not holding the microfibrils orientated so rigidly. Once this extent of movement has taken place the normal sliding apart of cellulose microfibrils takes place. Therefore the modulus of rupture is greater in the four year sprinkled wood.

The pattern of failures recorded also supports this theory. More brash failures were found in the sprinkled wood. Increased movement of the microfibrils before failure resulted in an increase in stored energy in the sprinkled wood. This energy, released at the moment of failure as the cellulose fibres parted, was enough to cause a brash failure. The type of failure found in half the brash failures recorded appeared in fact to be a combination, the failure starting as a simple fracture pattern but the energy release causing the lower tension break to continue into the compression mode with the final or overall fracture being classed as brash.

Loss of wood substance in four year sprinkled wood was also demonstrated by an increased uptake particularly of n-hexane. Sprinkled wood was shown to take up much more n-hexane than fresh wood. When treatment was with water only a small increase in uptake was found for sprinkled wood. A non polar liquid like n-hexane does not penetrate the cell walls and thus the movement of this liquid between cells

must be by pits. The large increase in uptake of the sprinkled wood emphasises the loss of torus and margo found by S.E.M.; the pits were open for movement of a liquid between cells. In fresh wood the torus and margo were intact and aspirated thus fewer paths of movement were available for n-hexane. Uptake of water was different. The difference found between sprinkled and fresh wood was slight. Bailey and Preston (1970) have shown that the annulus of the pit and not the pores in the margo constitute the major resistance to flow of water and Nicholas and Siau (1973) contend that water can break hydrogen bonds, which means aspirated pits in fresh wood could become deaspirated. Thus after two hour soaking the forces restricting flow in both sprinkled and fresh wood could have equalised. Preston (1974) has also argued for the existence of capillaries in cell walls which contribute to flow, particularly if some cell wall substance is lost. However when comparing soaking times of one and two hours, it was found that fresh wood was not affected, taking up a similar amount of water, while sprinkled wood took up more water after two hours' soaking. This suggests that with sprinkled wood, overcharging during commercial preservative treating could be experienced.

Various reasons have been advanced as to why water soaking is effective in preserving wood. Three theories which appeared to explain the mechanism were selected and their applicability to protecting *Pinus radiata* by intermittent water sprinkling were examined.

Oxygen levels found in the four year sprinkled wood were more than adequate to support the growth of most stain

and rot fungi. Published figures for a wide range of stain and some rot fungi showed the minimum oxygen levels required by these fungi were much lower than the levels found in the sprinkled wood. Carbon dioxide levels were also not inhibitory. The levels of oxygen and carbon dioxide found in the sprinkled wood were very close to the fresh wood figures pub lished by Thacker and Good (1952) and MacDougal and Working (1933). Oxygen levels found were lower than oxygen levels in air and carbon dioxide levels higher than air levels.

Temperature was not found to adversely affect the growth of fungi within the range tested. The temperatures recorded during the winter months of 1980 were higher than $5^0$C over most of the period recorded and laboratory work indicated that growth of two rot fungi can proceed at this temperature. The minimum temperature levels for growth of a wide range of stain and rot fungi, according to literature surveyed are also at or below $5^0$C.

Interactions investigated between bacteria and a rot fungus showed that bacteria established in wood prevented the spread and development of the fungus. Under continually wet conditions the fungus was quite capable of degrading sterilised wood but not wood inhabited by bacteria.

The mechanism by which *Pinus radiata* is protected by sprinkling was not explained in this experimental series but some leads were established. Oxygen was not limiting nor carbon dioxide inhibitory in four year sprinkled wood, so clearly the mechanism at this time did not include low oxygen levels. However this does not preclude this factor being

important in the first three years of sprinkling. It is significant that after four years some fungi were isolated with the potential to degrade wood, so possibly the mechanism which prevented early colonisation of the wood by fungi was no longer operating after four years.

Rot fungi were found after four years' sprinkling but not after three. It would seem from the evidence presented that three years constitutes a storage time in which changes to the wood structure do not preclude its use in most commercial areas. The area in which three year sprinkled wood might not be confidently used would be where it was subjected to permanent loadings, e.g. in beams. Hardness of the wood was not shown to be significantly affected. When preservative treating such wood, the increased uptake found would mean that reduced pumping times should be used to achieve the correct preservative loading.

## 8:1 SUMMARY OF CONCLUSIONS

1) Modulus of rupture and modulus of elasticity increased after four years' sprinkling with no increase in the work to maximum load. A possible explanation is given for the increase in modulus of rupture.

2) An increase in the incidence of brittle fracture in four year sprinkled wood was found.

3) Permeability to n-Hexane of wood sprinkled for four years increased. Water uptake by sprinkled wood increased slightly and was very dependent on the time of soaking.

4) The level of oxygen and carbon dioxide and the temperature found in the wood did not appear to be inhibitory to the development of rot fungi in the four year sprinkled wood.

5) Bacteria isolated from the wood were found to inhibit the growth of a selected rot fungus.

6) Sprinkling wood for four years did not render the wood less susceptible to sap stain organisms.

7) Almost all isolations of fungi from the logpile at Balmoral could be placed in the first stage of the successions worked out by Butcher (1968) and Bannerjee and Levy (1970).

8) After four years' sprinkling the appearance of a few rot organisms indicates that the succession could be beginning to change.

9) Bacteria isolated from the small logpile could not be adequately grouped using degradation of cellulose, pectin and starch as the major characters.

10) The bacterial succession found in the small logpile showed both gram negative and gram positive bacteria present although there was a predominance of gram negative organisms at any one time. Enterobacter and coryneform bacteria were the groups most frequently isolated.

11) After three years' sprinkling work with light, polarised light and scanning electron microscopy found no degraded ray cells and no margo or torus in the pits of the tracheid cells. Some damage to the $S_3$ wall layer was also noted.

## 8:2 RECOMMENDATIONS

1) Three years be considered as the maximum storage period in which little change will occur to adversely affect the use of the timber in the widest number of utilisation applications.

2) The timber sprinkled for three years be not used as beams or members that carry a dead load without some consideration being given to the brittleness found.

3) Revised preservative treatment schedules be developed to recognise the increased uptake with prolonged soaking.

4) Sapstain protection during stack drying be as adequate as for fresh timber.

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# A P P E N D I C E S

APPENDIX A1

## LOGPILE ARRANGEMENT

The logpile situated on the banks of the Hurunui river in Balmoral State Forest, consisting of eight rows of logs. Six of the rows were made up of 45-year-old *Pinus radiata* D Don; one row was a mixture of *P radiata* and Douglas fir and the final row was of *P radiata* and Douglas fir posts. On the end of the post row was 100 $m^3$ of older *P radiata* which was added to the pile after three years.

All work was carried out on the six rows of the *P radiata*. These rows were 150 m long and 4.5 m high. Log lengths were 12 m and 6-8 m, with some 4 m logs on top. Rows 2, 3, 4 and 6 had logs of 8 m on the bottom, with rows 2, 3 and 6 having logs of 4 m on top. Rows 1 and 5 had logs of 12 m long on the bottom and logs of 4 m on top.

Figure A1 outlines the arrangement of the logpile detailing the setting out of the spray lines in relation to the piles. The spray lines were arranged one between each pile to spray the ends of the logs, and a spray line over the top of each pile, except for the piles with 12 m long logs where two spray lines were set out over top.

The piles had the following volumes of timber at the start of the spraying:

| | | | |
|---|---|---|---|
| row 1 | 2720 $m^3$ | row 4 | 1530 $m^3$ |
| row 2 | 2600 $m^3$ | row 5 | 2950 $m^3$ |
| row 3 | 2230 $m^3$ | row 6 | 2610 $m^3$ |

APPENDIX A2

Wood samples were taken from Logpile numbers one to five. After three years sprinkling fifty random samples were taken over the whole of piles one to five. Each sample consisted of a disc of wood cut from the accessable portion of the log and being at least 100mm in thickness. From this disc a sapwood and heartwood sample of 100 x 50 x 50mm was taken. After four years sprinkling, twenty random samples were taken from log pile number one, the method of sampling being the same as above.

APPENDIX B1

SCHEDULE FOR STAINING SECTIONS FOR LIGHT MICROSCOPE

| Step | Treatment | Time |
|---|---|---|
| 1 | 50 percent alcohol | 15 minutes |
| 2 | 75 percent alcohol | 15 minutes |
| 3 | 85 percent alcohol | 15 minutes |
| 4 | 95 percent alcohol | 15 minutes |
| 5 | safrinin | 15 minutes |
| 6 | 95 percent alcohol | rinse |
| 7 | acid alcohol | rinse |
| 8 | 95 percent alcohol | rinse |
| 9 | absolute alcohol | 2 minutes |
| 10 | fast green | 5 minutes |
| 11 | oil of cloves | rinse |
| 12 | xylol | 2 minutes |
| 13 | xylol | 2 minutes |

All sections were taken directly from the microtome to the first stage of the staining schedule. Sections were handled with a camel hair brush.

STAINS

Safrinin: 2 percent safrinin in 95 percent alcohol

Fast Green: 1 percent fast green added to 1:3 clove oil/95 percent alcohol

note: alcohol is ethyl alcohol; wood was stored in formalin before sledge microtoming; sections were placed on a glass slide after staining and a cover slip placed over.

APPENDIX B2

## METHOD OF PREPARING SECTIONS FOR SCANNING ELECTRON MICROSCOPE

The method used for preparing specimens for viewing under S.E.M. was basically that of Exley, Butterfield and Meylan (1973). Samples to be sectioned for viewing were first soaked for 24 hours in formal acetic acid before cutting to expose the faces to be looked at. Every cut was made with an unused face of a razor blade. After final cutting the following drying schedule was followed:

| Step | Treatment | Time |
|---|---|---|
| 1 | 50 percent alcohol | 6 hours |
| 2 | 60 percent alcohol | 6 hours |
| 3 | 70 percent alcohol | 6 hours |
| 4 | 75 percent alcohol | 1 hour |
| 5 | 80 percent alcohol | 1 hour |
| 6 | 85 percent alcohol | 1 hour |
| 7 | 90 percent alcohol | 1 hour |
| 8 | 95 percent alcohol | 2 hours |
| 9 | 100 percent alcohol | 6 hours |
| 10 | | 24 hours |
| 11 | Critical point dry | approx. 6 hours |

After critical point drying the blocks were glued on to clean stubs using Araldite epoxy resin. The mounted stubs were stored in a dessicator over silica gel until viewed.

APPENDIX C1

# SAMPLING STRATEGIES

## Microbiological samples of Balmoral Logpile

### Nested Analysis of Variance

| Variation | df |
|---|---|
| Among Piles | 3 |
| Height within Piles | 8 |
| Depths Core within Heights | 24 |
| Depths Core Error | 294 |
| Total | 329 |

## Strength Test Samples, and n-Hexane Uptake

### Nested Analysis of Variance

| Variation | df |
|---|---|
| Among Piles | 1 |
| Heights within Piles | 2 |
| Depths within Heights | 28 |
| Total | 128 |

APPENDIX C1

## SAMPLING FROM SMALL LOGPILES

As outlined in the text the two logpiles set up contained bolts of *Pinus radiata* 2m long and averaging 150mm in diameter. The sprinkled pile contained $2m^3$ of timber and the unsprinkled pile contained $1m^3$ of timber. Samples were taken at weekly intervals for the first 15 weeks, every two weeks for the next four samples and the last sample was after four weeks. Sampling was random throughout the pile being samples both within pile and within logs. At each sample five samples were taken from the unsprinkled pile and ten from the sprinkled pile. Each sample consisted of an increment boring as in Appendix F1 except that only sapwood discs were examined. Tests applied were those outlined in Appendix F1 and F5.

APPENDIX C2

## UPTAKE OF n-HEXANE

The solvent n-Hexane was obtained from Shell Chemical Company. Wood from the logpile or from fresh wood from a similar stand to the wood in the logpile was broken down to sticks of 40 x 40 x 1000 mm. These were kiln dried using a slow schedule followed by four months' conditioning at 20$^0$C and 60 percent relative humidity. The sticks were then machined to their final size of 20 x 20 x 20 mm and conditioned for another two months in conditions the same as above. The procedure followed was:

1) Each block was weighed within the room at 20$^0$C and 60 percent relative humidity.
2) Immediately after weighing each block was immersed in n-Hexane for 15 seconds,
3) Aired dried for 15 seconds,
4) Reweighed.

APPENDIX C3

3 LEVEL NESTED ANOVA TRANSFORMATION CODE = 0

ANOVA TABLE

| LEVEL | SS | DF | MS | FS |
|---|---|---|---|---|
| 3 | 311.838 | 1. | 311.8385 | 3.4297 |
| 2 | 181.846 | 2.<br>2.0 | 90.9228<br>90.9228 | 1.8488 |
| 1 | 1377.009 | 28.<br>28.0 | 49.1789<br>49.1789 | 0.5642 |
| 0 | 11156.880 | 128. | 87.1631 | |

VARIANCE COMPONENTS

| LEVEL | VAR.COMP. | PERCENT |
|---|---|---|
| 3 | 2.2214 | 2.6819 |
| 2 | 1.0436 | 1.2599 |
| 1 | -7.5968 | -9.1715 |
| 0 | 87.1631 | 105.2297 |

TABLE OF COEFFICIENTS

| | | | |
|---|---|---|---|
| 3 | 5.0 | 40.0 | 80.0 |
| 2 | 5.0 | 40.0 | |
| 1 | 5.0 | | |

APPENDIX C4

Analysis of n-Hexane Uptake in Sprinkled and Fresh Wood

ANOVA of n-Hexane Uptake

| | df | ss | ms | F |
|---|---|---|---|---|
| Treatments | 1 | 62376.1414 | 62376.1414 | 18.7992 * * |
| Error | 151 | 501021.1453 | 3318.0208 | |
| Total | 152 | 563397.2868 | 3716.5611 | |

Regression of Density and n-Hexane Uptake

| Analysis of Covariance | | | | Deviations from Regression | | | |
|---|---|---|---|---|---|---|---|
| | df | ss | ms | df | ss | ms | F |
| Treatments | 1 | 62376.14 | 62376.14 | 1 | 66765.51 | 66765.51 | 22.42 * * |
| Error | 151 | 501021.14 | 3318.02 | 150 | 446725.14 | 2978.17 | |
| Total | 152 | 563397.28 | 3716.56 | | | | |

Test Difference of Regression Slopes

| | df | ss | ms | F |
|---|---|---|---|---|
| Between | 1 | 37541.4136 | 37541.4136 | 13.6703 * * |
| Within | 149 | 409183.7233 | 2746.1994 | |

APPENDIX C4 (continued)

Regression of Ring Number per 2 cm and n-Hexane Uptake

| | Analysis of Covariance | | | Deviations from Regression | | | |
|---|---|---|---|---|---|---|---|
| | df | ss | ms | df | ss | ms | F |
| Treatment | 1 | 62376.14 | 62376.14 | 1 | 40236.28 | 40236.28 | 13.27 * * |
| Error | 151 | 501021.15 | 3318.02 | 150 | 454809.14 | 3032.06 | |
| Total | 152 | 563397.28 | 3716.56 | | | | |

Test Difference of Regression Slopes

| | df | ss | ms | F |
|---|---|---|---|---|
| Between | 1 | 8748.426 | 8748.426 | 2.92 ns |
| Within | 149 | 446060.711 | 2993.696 | |

* * significant at 1 percent

ns not significant

APPENDIX C5

Analysis of Water Uptake in Sprinkled and Fresh Wood

ANOVA of Water Uptake

| | df | ss | ms | F |
|---|---|---|---|---|
| Treatments | 1 | 464733.051 | 464733.051 | 11.333 * * |
| Error | 151 | 6192060.602 | 41007.024 | |
| Total | 152 | 6656793.653 | 43794.695 | |

Regression of Density and Water Uptake

| | Analysis of Covariance | | | Deviations from Regression | | | |
|---|---|---|---|---|---|---|---|
| | df | ss | ms | df | ss | ms | F |
| Treatments | 1 | 464733.051 | 464733.05 | 1 | 509469.28 | 509469.28 | 14.02 * * |
| Error | 151 | 6192060.602 | 41007.02 | 150 | 5452234.98 | 36348.23 | |
| Total | 152 | 6656793.653 | 43794.69 | | | | |

Test Difference of Regression Slopes

| | df | ss | ms | F |
|---|---|---|---|---|
| Between | 1 | 10530.169 | 10530.169 | 0.288 ns |
| Within | 149 | 5441704.814 | 36521.509 | |

APPENDIX C5 (continued)

Regression of n-Hexane Uptake and Water Uptake

| | Analysis of Covariance | | | Deviations from Regression | | | |
|---|---|---|---|---|---|---|---|
| | df | ss | ms | df | ss | ms | F |
| Treatments | 1 | 464733.051 | 464733.051 | 1 | 87665.01 | 87665.01 | 2.575 ns |
| Error | 151 | 6192060.602 | 41007.0239 | 150 | 5105835.91 | 34038.91 | |
| Total | 152 | 6656793.653 | 43794.6951 | | | | |

Test Difference of Regression Slopes

| | df | ss | ms | F |
|---|---|---|---|---|
| Between | 1 | 1588484.540 | 1588484.540 | 67.29 * * |
| Within | 149 | 3517351.253 | 23606.384 | |

* * 1 percent significance

ns not significant

# APPENDIX D1

3 LEVEL NESTED ANOVA TRANSFORMATION CODE = 0

ANOVA TABLE

| LEVEL | SS | DF | MS | FS |
|---|---|---|---|---|
| 3 | 13754.961 | 1. | 13754.9609 | 26.7799 * |
| 2 | 1025.350 | 2.<br>1.9 | 512.6750<br>513.6299 | 1.8841 |
| 1 | 7625.265 | 28.<br>27.5 | 272.3309<br>272.1120 | 0.9016 |
| 0 | 35340.621 | 117. | 302.0566 | |

VARIANCE COMPONENTS

| LEVEL | VAR.COMP. | PERCENT |
|---|---|---|
| 3 | 176.9316 | 36.9324 |
| 2 | 6.4727 | 1.3511 |
| 1 | -6.3916 | -1.3342 |
| 0 | 302.0566 | 63.0507 |

TABLE OF COEFFICIENTS

| | | | |
|---|---|---|---|
| 3 | 4.7 | 37.3 | 74.5 |
| 2 | 4.7 | 37.2 | |
| 1 | 4.7 | | |

3 LEVEL NESTED ANOVA TRANSFORMATION CODE = 0

ANOVA TABLE

| LEVEL | SS | DF | MS | FS |
|---|---|---|---|---|
| 3 | 21980.099 | 1. | 21980.0991 | 43.3018 |
| 2 | 1016.544 | 2.<br>116 | 508.2722<br>507.6027 | 0.5503 |
| 1 | 25803.435 | 28.<br>27.7 | 921.5512<br>923.6053 | 1.4525 |
| 0 | 74230.209 | 117. | 634.4462 | |

VARIANCE COMPONENTS

| LEVEL | VAR.COMP. | PERCENT |
|---|---|---|
| 3 | 292.8365 | 29.9475 |
| 2 | -11.1767 | -1.1430 |
| 1 | 61.7331 | 6.3132 |
| 0 | 634.4462 | 64.8823 |

TABLE OF COEFFICIENTS

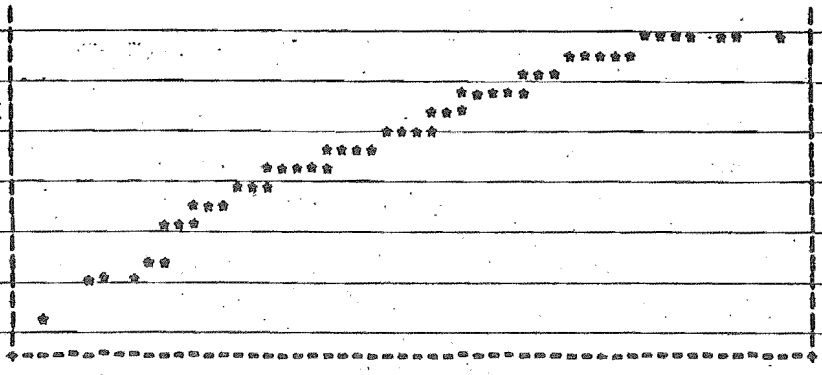
| | | | |
|---|---|---|---|
| 3 | 4.7 | 37.3 | 74.5 |
| 2 | 4.7 | 37.2 | |
| 1 | 4.7 | | |

APPENDIX D2

MODULOUS OF RUPTURE

DISTRIBUTION PLOT:

FOR A NORMAL DISTRIBUTION THIS SHOULD BE A STRAIGHT LINE



GRAND MEAN = 84.795 UNWEIGHTED MEAN = 80.569 COEFFICIENT OF VARIATION = 18.86%

ANALYSIS OF VARIANCE

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| T | 2203.49147 | 1 | 2203.4914714 | 9.2603 | 0.002763 |
| ERROR | 35930.06772 | 151 | 237.9514418 | | |
| TOTAL | 38134.15919 | 152 | 250.8826262 | | |

FACTOR T

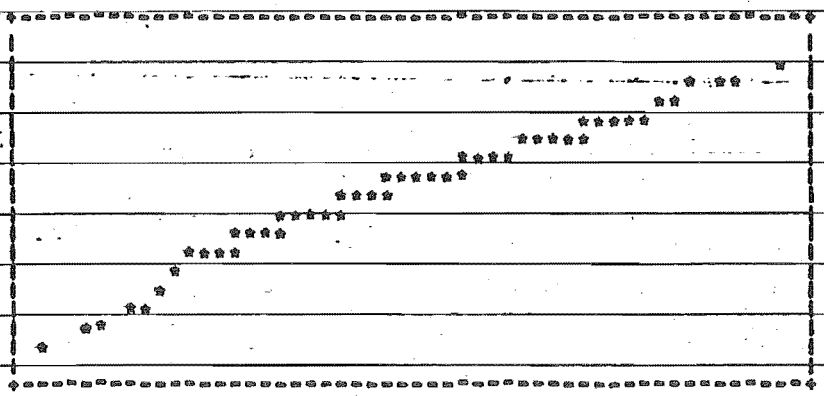
| | MEAN | MS | SD | SE OF MEAN VARYING ERROR | SE OF MEAN CONSTANT ERROR |
|---|---|---|---|---|---|
| T= | 76.562 | 228.36486052 | 15.11174578 | 2.07575792 | 2.11687938 |
| | 89.557 | 242.98681789 | 15.58803445 | 1.55080344 | 1.54250748 |

DO THE 2 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
VARIANCE-RATIO TEST : F = 1.0640, D.F. = 52, 99, P = 0.778450

MODULOUS OF ELASTICITY

DISTRIBUTION PLOT

FOR A NORMAL DISTRIBUTION THIS SHOULD BE A STRAIGHT LINE



GRAND MEAN = 8806.3  UNWEIGHTED MEAN = 8799.1  COEFFICIENT OF VARIATION = 22.34%

ANALYSIS OF VARIANCE

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| T | 11683013.5 | 1 | 11683013.500 | 2.9620 | 0.087291 |
| ERROR | 595591604.5 | 151 | 3944315.261 | | |
| TOTAL | 607274618.0 | 152 | 3995227.750 | | |

TEDDYBEAR # INSTRON # E

FACTOR T

| | MEAN | MS | SD | SE OF MEAN VARYING ERROR | SE OF MEAN CONSTANT ERROR |
|---|---|---|---|---|---|
| T= | 8508.7 | 2967590.2727 | 1722.6695193 | 236.62065667 | 272.80220430 |
| * | 9089.4 | 4457342.5284 | 2111.2419398 | 211.12419398 | 196.60306253 |

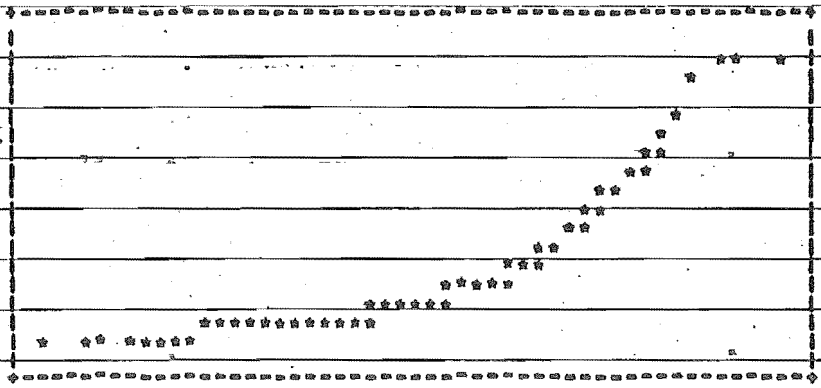
DO THE 2 LEVELS HAVE DIFFERENT ERROR VARIANCE ?

VARIANCE-RATIO TEST : F = 1.5020, D.F. = 52, 99, P = 0.003739

WORK TO MAXIMUM LOAD

DISTRIBUTION PLOT:

FOR A NORMAL DISTRIBUTION THIS SHOULD BE A STRAIGHT LINE



GRAND MEAN = 0.13748 UNWEIGHTED MEAN = 0.13914 COEFFICIENT OF VARIATION = 53.38%

ANALYSIS OF VARIANCE

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| T | 0.00408000825 | 1 | .00408008249 | 0.7576 | 0.385458 |
| ERROR | 0.8132028928 | 151 | .00538544962 | | |
| TOTAL | 0.8172829753 | 152 | .00537686168 | | |

TEDDYBEAR : INSTRON : N

FACTOR T

| | MEAN | MS | SD | SE OF MEAN VARYING ERROR | SE OF MEAN CONSTANT ERROR |
|---|---|---|---|---|---|
| T= | 0.14457 | 0.00562624 | 0.07500827 | 0.01030318 | 0.01000029 |
| * | 0.13372 | 0.00525897 | 0.07251878 | 0.00725188 | 0.00730856 |

DO THE 2 LEVELS HAVE DIFFERENT ERROR VARIANCE ?

VARIANCE-RATIO TEST : F= 1.0698, D.F. = 52, 99, P = 0.741220

## REGRESSION OF BENDING STRENGTH AND DENSITY

TEDDYBEAR : INSTRON : D R

ANALYSIS OF COVARIANCE = ARE THE ADJUSTED MEANS (& HENCE THE INTERCEPTS) SIGNIFICANTLY AFFECTED BY TREATMENT FACTORS ?
THE TESTS ASSUME THAT THE SLOPES ARE THE SAME (SEE BELOW)

| | SS | DF | MS | DEVIATIONS FROM REGRESSION SS | DF | MS | F | P |
|---|---|---|---|---|---|---|---|---|
| T | 2203.49147 | 1 | 2203.4914714 | 2696.51463 | 1 | 2696.5146313 | 22.0669 | 0.000006 |
| ERROR | 35930.66772 | 151 | 237.9514418 | 18327.93482 | 150 | 122.1862321 | | |
| TOTAL | 38134.15919 | 152 | 250.8826262 | | | | | |

FACTOR T

| | DF | INTERCEPT | SE | P | SLOPE | SE | P |
|---|---|---|---|---|---|---|---|
| T= | 51 | -25.24140822 | 9.60163942 | 0.011293 | 225.9807895 | 21.1519847 | 0.000000 |
| ◆ | 98 | -8.875684064 | 11.8266239 | 0.454762 | 209.1917483 | 26.3374815 | 0.000000 |

ARE THE SLOPES DIFFERENT ? = A TEST TO SEE WHETHER THE REGRESSION SLOPES WITHIN THE 2 LEVELS ARE SIGNIFICANTLY DIFFERENT, BY COMPARING THE BETWEEN-LEVEL DEVIATIONS FROM REGRESSION WITH THE WITHIN-LEVEL DEVIATIONS

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| BETWEEN | 25.93840 | 1 | 25.9383996 | 0.2112 | 0.646522 |
| WITHIN | 18301.99642 | 149 | 122.8321907 | | |

| ADJUSTED MEANS | MS | SD | SE OF MEAN VARYING ERROR | SE OF MEAN CONSTANT ERROR |
|---|---|---|---|---|
| T= 76.155 | 70.81093187 | 8.41492316 | 1.15587859 | 1.51035457 |
| ◆ 64.984 | 147.93703394 | 12.16293690 | 1.21629369 | 1.10537881 |

DO THE 2 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
VARIANCE-RATIO TEST : F = 2.0892, D.F. = 52, 99, P = 0.001677

JOINT RESIDUAL (WITHIN-TREATMENT) VARIATION

REGRESSION EQUATION :

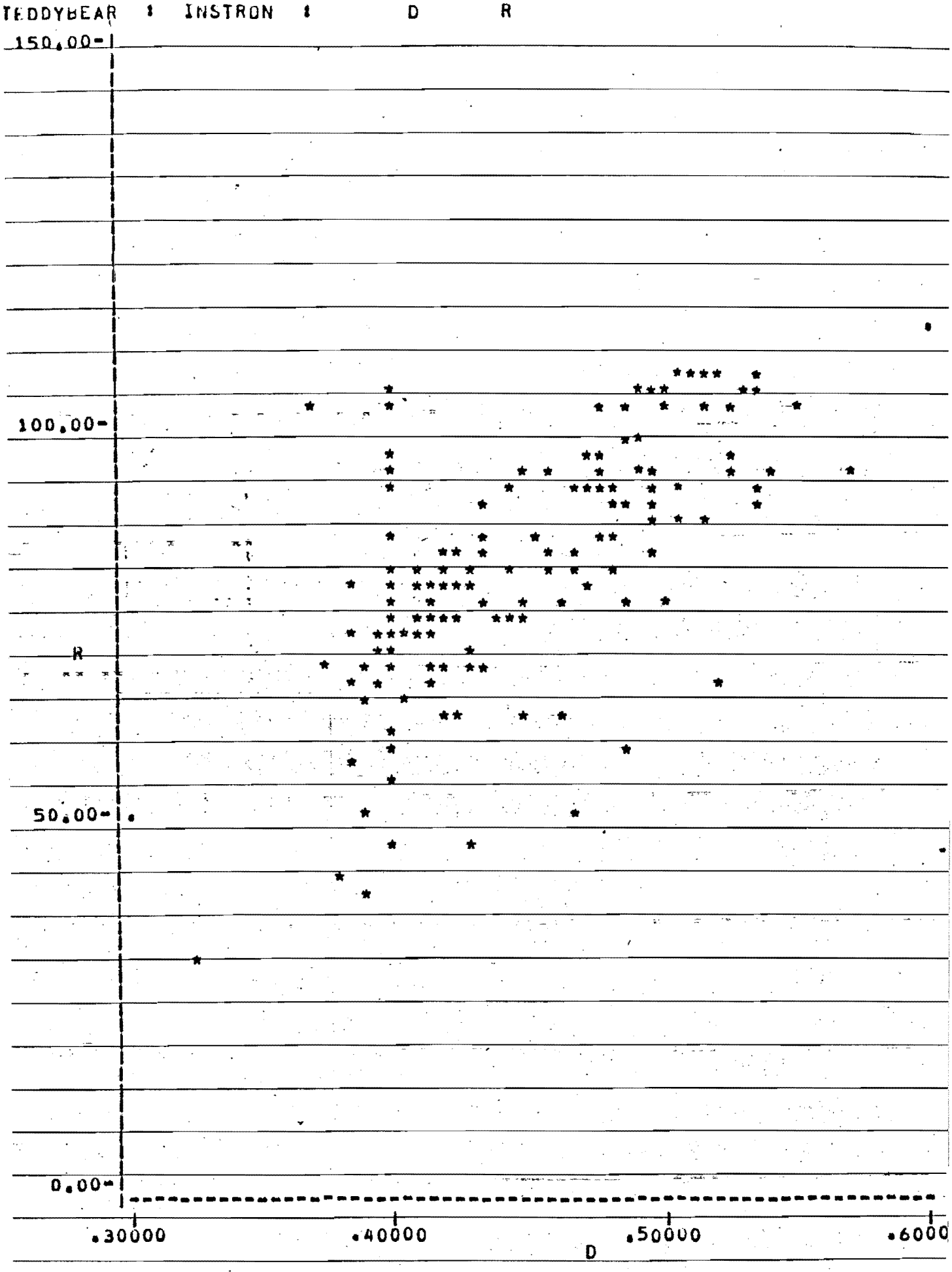
R = -97.065040191 + 216.36818164 ◆ 0

| | | |
|---|---|---|
| SE | 8.1361607889 | 18.026627390 |
| PROB | 0.000000 | 0.000000 |

IS THE WHOLE REGRESSION SIGNIFICANT ?

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| REGRESSION | 17602.73290 | 1 | 17602.732901 | 144.0648 | 0.000000 |
| DEVIATIONS | 18327.93482 | 150 | 122.186232 | | |
| TOTAL | 35930.66772 | 151 | 237.951442 | | |

THE REGRESSION ACCOUNTS FOR 48.991 % OF THE VARIATION (SS)


TEDDYBEAR : INSTRON : D R
150.00-
100.00-
R
50.00-
0.00-
.30000
.40000
.50000
.6000
D

## REGRESSION OF MODULOUS OF ELASTICITY AND DENSITY

ANALYSIS OF COVARIANCE = ARE THE ADJUSTED MEANS (& HENCE THE INTERCEPTS) SIGNIFICANTLY AFFECTED BY TREATMENT FACTORS ?
THE TESTS ASSUME THAT THE SLOPES ARE THE SAME (SEE BELOW)

| | SS | DF | MS | DEVIATIONS FROM REGRESSION SS | DF | MS | F | P |
|---|---|---|---|---|---|---|---|---|
| T | 11683013.5 | 1 | 11683013.500 | 15882569.9 | 1 | 15882569.881 | 6.4580 | 0.012061 |
| ERROR | 595591604.5 | 151 | 3944315.261 | 368903550.5 | 150 | 2459357.003 | | |
| TOTAL | 607274618.0 | 152 | 3995227.750 | | | | | |

FACTOR T

| | DF | INTERCEPT | SE | P | SLOPE | SE | P |
|---|---|---|---|---|---|---|---|
| T= | 51 | -1886.098293 | 1314.90014 | 0.157561 | 23067.63513 | 2896.66653 | 0.000000 |
| * | 98 | -2372.067656 | 1691.89571 | 0.164070 | 25661.72125 | 3767.79309 | 0.000000 |

ARE THE SLOPES DIFFERENT ? = A TEST TO SEE WHETHER THE REGRESSION SLOPES WITHIN THE 2 LEVELS ARE SIGNIFICANTLY DIFFERENT, BY COMPARING THE BETWEEN-LEVEL DEVIATIONS FROM REGRESSION WITH THE WITHIN-LEVEL DEVIATIONS

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| BETWEEN | 618287.2 | 1 | 618287.186 | 0.2501 | 0.617711 |
| WITHIN | 368285263.3 | 149 | 2471713.176 | | |

| ADJUSTED MEANS | | MS | SD | SE OF MEAN VARYING ERROR | SE OF MEAN CONSTANT ERROR |
|---|---|---|---|---|---|
| T= | 8460.3 | 1329442.4085 | 1153.0144876 | 158.37072026 | 215.41346829 |
| * | 9137.9 | 3028005.5072 | 1740.1165212 | 174.01165212 | 156.82337208 |

DO THE 2 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
VARIANCE-RATIO TEST : F = 2.2777, D.F. = 52, 99, P = 0.000442

TEDDYBEAR : INSTRON : D E

JOINT RESIDUAL (WITHIN-TREATMENT) VARIATION

REGRESSION EQUATION :

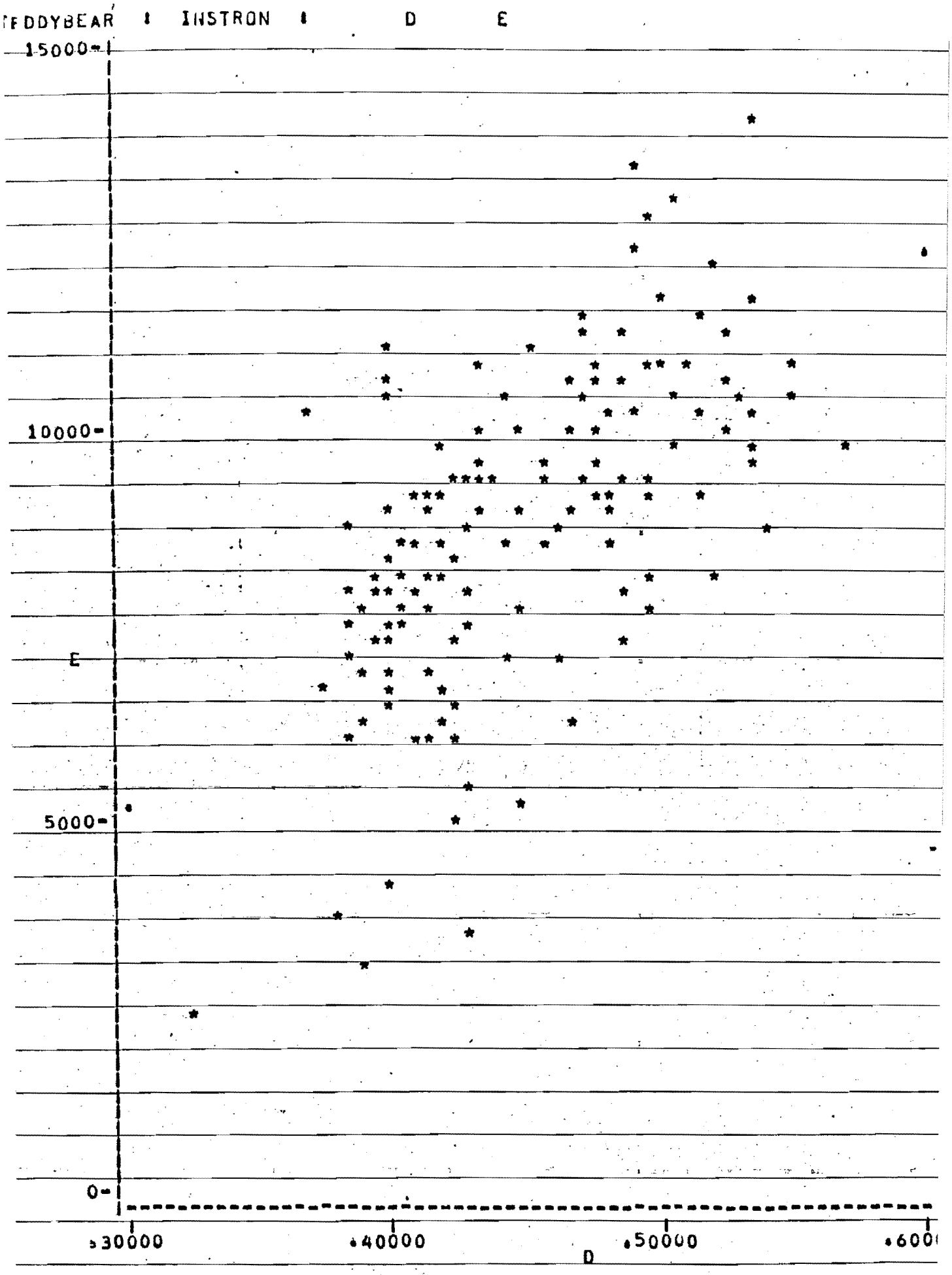
E = -11015.066066 +24553.740570 * D

| | | |
|---|---|---|
| SE | 1154.3012723 | 2557.4911156 |
| PROB | 0.000000 | 0.000000 |

Is THE WHOLE REGRESSION SIGNIFICANT ?

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| REGRESSION | 226688054.0 | 1 | 226688054.01 | 92.1737 | 0.000000 |
| DEVIATIONS | 368903550.5 | 150 | 2459357.00 | | |
| TOTAL | 595591604.5 | 151 | 3944315.26 | | |

THE REGRESSION ACCOUNTS FOR 38.061 % OF THE VARIATION (SS)

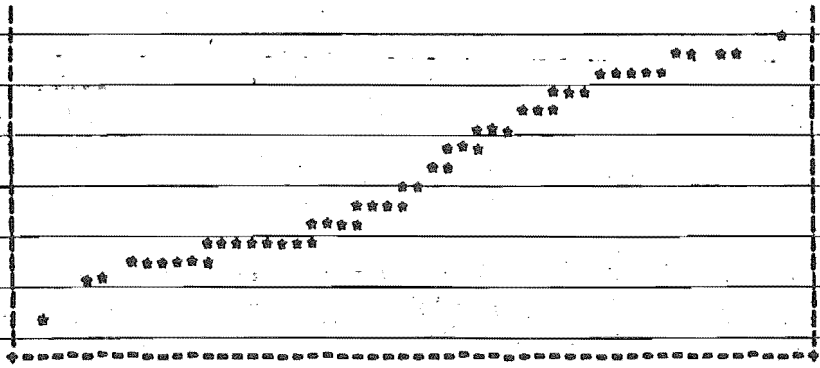

TEDDYBEAR * INSTRON * D E
15000-
10000-
E
5000-
0-
30000
40000
50000
600
D

APPENDIX D5

ANALYSIS OF DENSITY

DISTRIBUTION PLOT

FOR A NORMAL DISTRIBUTION THIS SHOULD BE A STRAIGHT LINE



GRAND MEAN = 0.44800 UNWEIGHTED MEAN = 0.44861 COEFFICIENT OF VARIATION = 11.14%

ANALYSIS OF VARIANCE

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| T | 0.0005391165 | 1 | .00053911648 | 0.2165 | 0.642387 |
| ERROR | 0.3760047303 | 151 | .00249009755 | | |
| TOTAL | 0.3765438468 | 152 | .00247726215 | | |

TEDDYBEAR : INSTRON : D

FACTOR T

| | MEAN | MS | SD | SE OF MEAN VARYING ERROR | SE OF MEAN CONSTANT ERROR |
|---|---|---|---|---|---|
| T= | 0.45058 | 0.00309081 | 0.05559508 | 0.00763657 | 0.00683441 |
| * | 0.44664 | 0.00217457 | 0.04663229 | 0.00466323 | 0.00494009 |

DO THE 2 LEVELS HAVE DIFFERENT ERROR VARIANCE ?

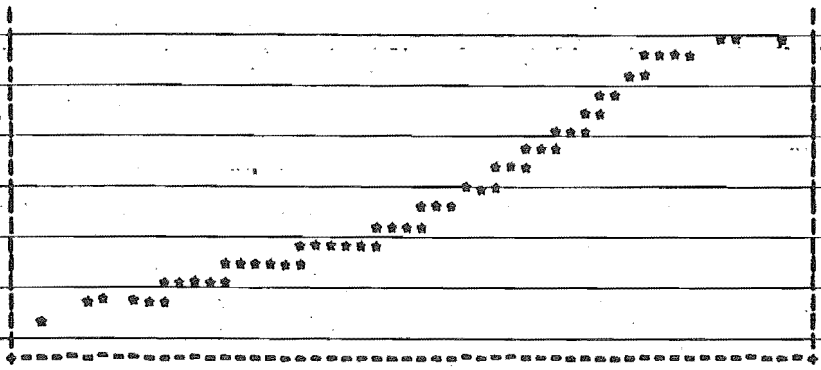
VARIANCE-RATIO TEST : F = 1.4213, D.F. = 52, 99, P = 0.134890

APPENDIX D6

ANALYSIS OF HARDNESS

DISTRIBUTION PLOT:

FOR A NORMAL DISTRIBUTION THIS SHOULD BE A STRAIGHT LINE



GRAND MEAN = 3.0392 UNWEIGHTED MEAN = 3.0173 COEFFICIENT OF VARIATION = 20.178

ANALYSIS OF VARIANCE

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| T | 0.70121015 | 1 | 0.7012101491 | 1.8656 | 0.174012 |
| ERROR | 56.75555794 | 151 | 0.3758646221 | | |
| TOTAL | 57.45676809 | 152 | 0.3780050532 | | |

TEDDYBEAR : INSTRON : H

FACTOR T

| | MEAN | MS | SD | SE OF MEAN VARYING ERROR | SE OF MEAN CONSTANT ERROR |
|---|---|---|---|---|---|
| T= | 2.9462 | 0.45967590 | 0.67799403 | 0.09312964 | 0.08421274 |
| + | 3.0864 | 0.33184253 | 0.57605775 | 0.05760578 | 0.06130780 |

DO THE 2 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
VARIANCE-RATIO TEST : F = 1.3852, D.F. = 52, 99, P = 0.105715

## REGRESSION OF HARDNESS AND DENSITY

ANALYSIS OF COVARIANCE = ARE THE ADJUSTED MEANS (& HENCE THE INTERCEPTS) SIGNIFICANTLY AFFECTED BY TREATMENT FACTORS ?
THE TESTS ASSUME THAT THE SLOPES ARE THE SAME (SEE BELOW)

| | SS | DF | MS | DEVIATIONS FROM REGRESSION SS | DF | MS | F | P |
|---|---|---|---|---|---|---|---|---|
| T | 0.70121015 | 1 | 0.7012101491 | 1.07769254 | 1 | 1.0776925387 | 5.6834 | 0.018377 |
| ERROR | 56.27555794 | 151 | 0.3758646221 | 28.44308143 | 150 | 0.1896205429 | | |
| TOTAL | 57.45676809 | 152 | 0.3780050532 | | | | | |

FACTOR T

| | DF | INTERCEPT | SE | P | SLOPE | SE | P |
|---|---|---|---|---|---|---|---|
| T= | 51 | -1.665163792 | 0.42156483 | 0.000241 | 10.23414448 | 0.92868858 | 0.000000 |
| * | 98 | -0.268166666 | 0.44471812 | 0.547899 | 7.5152822322 | 0.99037183 | 0.000000 |

ARE THE SLOPES DIFFERENT ? = A TEST TO SEE WHETHER THE REGRESSION SLOPES WITHIN THE 2 LEVELS ARE SIGNIFICANTLY DIFFERENT, BY COMPARING THE BETWEEN-LEVEL DEVIATIONS FROM REGRESSION WITH THE WITHIN-LEVEL DEVIATIONS

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| BETWEEN | 0.68024566 | 1 | 0.6802456623 | 3.6508 | 0.057963 |
| WITHIN | 27.78283577 | 149 | 0.1863277568 | | |

| ADJUSTED MEANS | | MS | SD | SE OF MEAN VARYING ERROR | SE OF MEAN CONSTANT ERROR |
|---|---|---|---|---|---|
| T= | 2.9291 | 0.14344115 | 0.37873626 | 0.05202343 | 0.05981426 |
| * | 3.1056 | 0.21196103 | 0.46039225 | 0.04603923 | 0.04354544 |

DO THE 2 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
VARIANCE-RATIO TEST : F = 1.4777, D.F. = 52, 99, P = 0.096910

TEDDYBEAR : INSTRON : D H

JOINT RESIDUAL (WITHIN-TREATMENT) VARIATION

REGRESSION EQUATION :

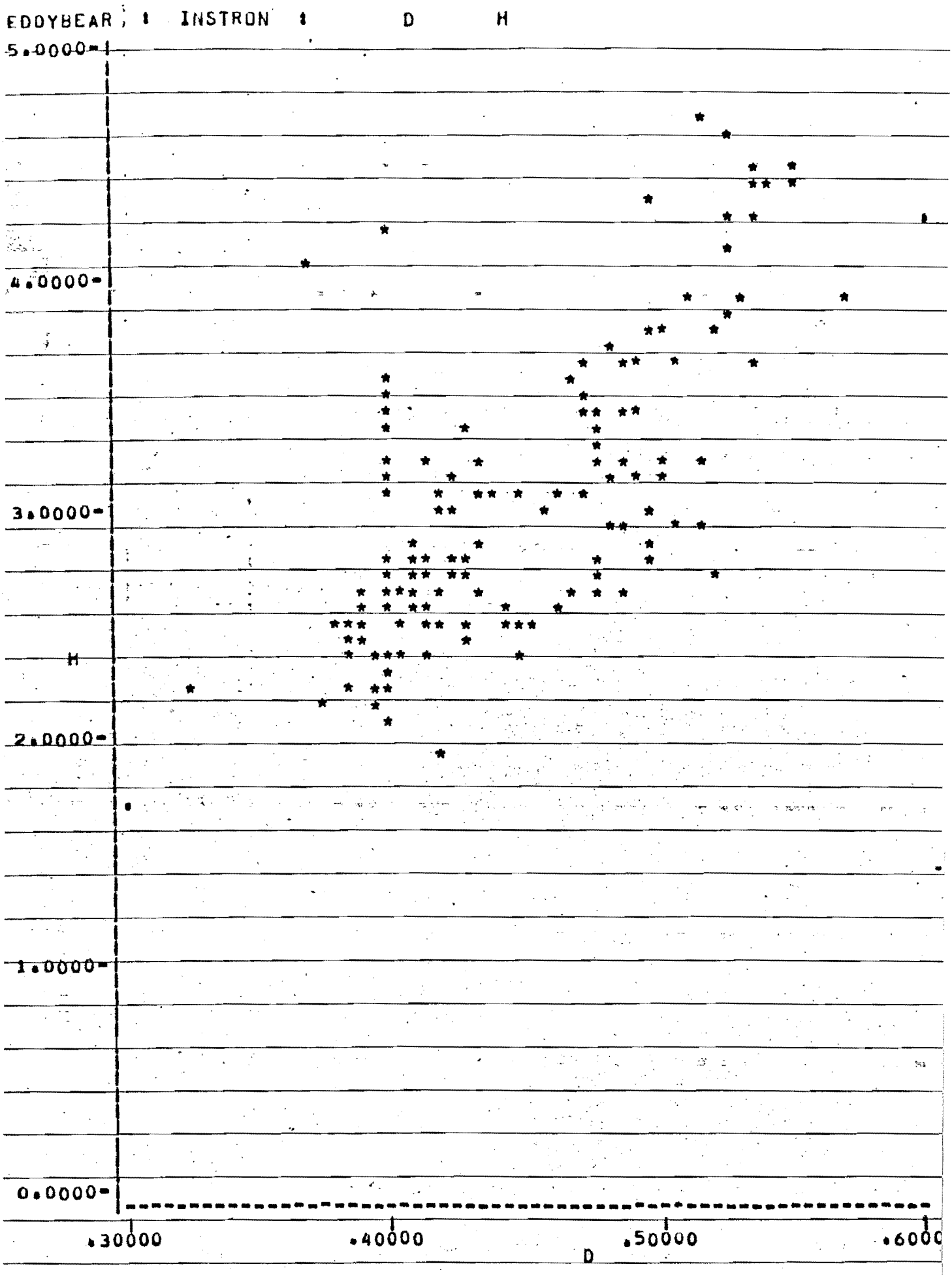
H = -3.8927966572 +8.6774531031 * D

| | | |
|---|---|---|
| SE | 0.3305170074 | 0.7101A33728 |
| PROB | 0.000000 | 0.000000 |

IS THE WHOLE REGRESSION SIGNIFICANT ?

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| REGRESSION | 28.31247651 | 1 | 28.312476512 | 149.3112 | 0.000000 |
| DEVIATIONS | 28.44308143 | 150 | 0.1896205429 | | |
| TOTAL | 56.75555794 | 151 | 0.3758864622 | | |

THE REGRESSION ACCOUNTS FOR 49.885 % OF THE VARIATION (SS)


EDDYBEAR ; 1 INSTRON 1 D H
5.0000
4.0000
3.0000
H
2.0000
1.0000
0.0000
.30000
.40000
.50000
.6000
D

## REGRESSION OF HARDNESS SQUARED AND DENSITY

ANALYSIS OF COVARIANCE = ARE THE ADJUSTED MEANS (& HENCE THE INTERCEPTS) SIGNIFICANTLY AFFECTED BY TREATMENT FACTORS ?
THE TESTS ASSUME THAT THE SLOPES ARE THE SAME (SEE BELOW)

| | | | | DEVIATIONS - FROM REGRESSION | | | | |
|---|---|---|---|---|---|---|---|---|
| | SS | DF | MS | SS | DF | MS | F | P |
| T | 18.7698462 | 1 | 18.769846177 | 1.5615196 | 1 | 1.561519601 | 8.4698 | 0.004165 |
| ERROR | 2479.5081222 | 151 | 16.420583591 | 27.4700409 | 149 | 0.184362691 | | |
| TOTAL | 2498.2779684 | 152 | 16.436039266 | | | | | |

FACTOR T

| VARIATES | | | | | D | | | H | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DF | INTERCEPT | SE | P | SLOPE | SE | P | SLOPE | SE | P |
| T= | 50 | -9.729735669 | 0.53009256 | 0.000000 | -3.783738374 | 1.8790253 | 0.049442 | 6.980434472 | 0.15407889 | 0.000000 |
| ♦ | 97 | -9.750965586 | 0.41415915 | 0.000000 | -1.184092295 | 1.1599638 | 0.309888 | 6.523296732 | 0.09389991 | 0.000000 |

ARE THE SLOPES DIFFERENT ? = A TEST TO SEE WHETHER THE REGRESSION SLOPES WITHIN THE 2 LEVELS ARE SIGNIFICANTLY DIFFERENT, BY COMPARING THE BETWEEN-LEVEL DEVIATIONS FROM REGRESSION WITH THE WITHIN-LEVEL DEVIATIONS

| | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| BETWEEN | 1.3801053 | 2 | 0.690052646 | 3.8880 | 0.022626 |
| WITHIN | 26.0899356 | 147 | 0.177482555 | | |

| TEDDYBEAR | INSTRON | D | H 2 | | |
|---|---|---|---|---|---|
| ADJUSTED MEANS | MS | SD | SE OF MEAN VARYING ERROR | SE OF MEAN CONSTANT ERROR | |
| T= | 9.6072 | 0.17875446 | 0.42279363 | 0.05607517 | 0.05697916 |
| ♦ | 9.3907 | 0.18358393 | 0.42846695 | 0.04284670 | 0.04293748 |

DO THE 2 LEVELS HAVE DIFFERENT ERROR VARIANCE ?
VARIANCE-RATIO TEST : F = 1.0270, D.F. = 52, 99, P = 0.892598

APPENDIX E1

MOISTURE CONTENT AND DENSITY OF BALMORAL FRESH AND SPRINKLED WOOD

F test of population variance $H_o : s_1^2 = s_1^2$

Sapwood

density sprinkled and unsprinkled F = 1.09 ns

moisture content sprinkled and unsprinkled F = 5.7 significant 5%

saturation sprinkled and unsprinkled F = 6.9 significant 5%

Heartwood

density sprinkled and unsprinkled F = 0.64 ns

moisture content sprinkled and unsprinkled F = 1.87 ns

saturation sprinkled and unsprinkled F = 0.66 ns

APPENDIX E2

## SAPSTAIN ORGANISMS

A mixed inoculum of sapstain and mould organisms was used. The cultures were obtained from F.R.I. Rotorua as malt agar slopes. These were:

*Ceratocystis spp*

*Diplodea pinea*

*Alternaria spp*

*Penicillium spp*

*Trichoderma viridae*

The above cultures were grown on malt agar petri dishes until profuse mycellium covered the surface (about two weeks). This mycellium was scraped off aseptically, placed in one litre of sterile water (all fungi into the one litre) and blended for one minute. The resultant slurry was used as the mixed inoculum. Inoculation was by spraying the wood until run off.

APPENDIX F1

# ISOLATION METHODS

All samples for bacterial and fungal isolations were obtained using an increment borer which removed a core 5 mm in diameter. The procedure for sampling was:

1) Clear bark to expose outer cambial layer
2) Flood surface of area to be sampled with ethyl alcohol
3) Insert increment borer which had been sterilized by dipping in ethyl alcohol
4) Remove core and place in sterile plastic bag
5) Place in cool container for transport to laboratory

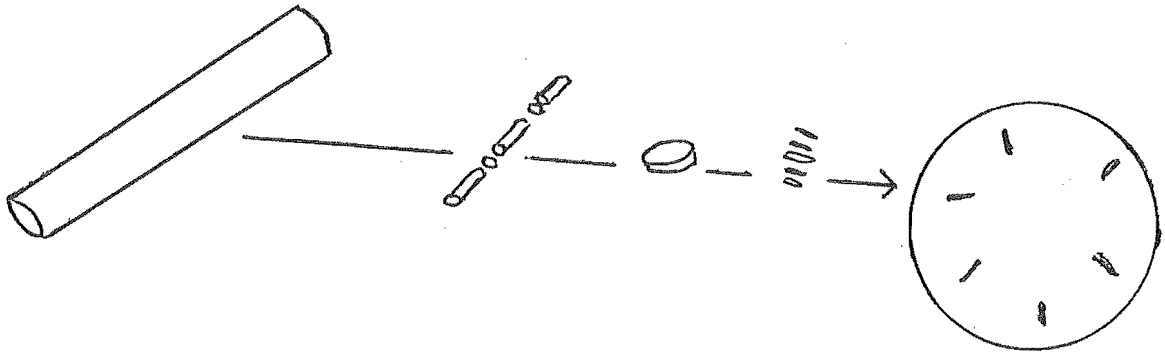
In the laboratory all sub-sampling was done in a sterile laminar flow cabinet. The core was sectioned using a flamed scalpel and forceps. All sample sizes were such that sectioning and placing on the various growth media was done the same day.

## Fungal Isolation

For isolation of fungi, discs 2 mm thick were cut from the core using flamed scalpel and forceps in a laminar flow cabinet. Each disc was further sectioned into six segments and these were spaced out round a petri dish. The medium used was:

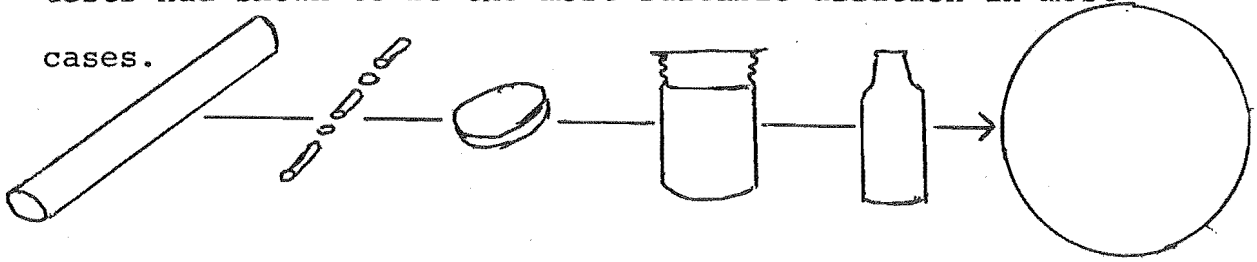
| | |
|---|---|
| malt | 25 g |
| streptomycin sulphate | 2 g |
| agar | 15 g |
| water | 1000 ml |

Initial identification and subculturing were carried out before any resultant growth became confluent. Identification was mainly by direct observation and water mounts.



Bacterial Isolation

For bacterial isolation discs 2 mm thick were cut from the core using flamed scalpel and forceps in a laminar flow cabinet. The disc was transferred to 5 ml of sterile 0.1 percent peptone water in a wide mouthed universal bottle. The disc was then macerated in the bottle by inserting the flamed mixing end of a high speed blender. The disc was comminuted for two fifteen second periods, ensuring that the peptone water did not heat. Earlier trials had shown that this was sufficient to break up the disc into a fibrous slurry. Serial dilutions were made and 0.2 ml of each 1:100 dilution was spread onto a 1 percent Difco nutrient agar plate; five plates were made for each 1:100 dilution, which tests had shown to be the most suitable dilution in most cases.



Log Core Disc 0.1% peptone + maceration Serial dilution Petri dish

All bacterial plates were incubated at 25$^0$C for seven days before counts and isolations were made. Colonies were picked off and transferred on to fresh nutrient agar plates, streaks made. Once grown the streak was examined for purity; this streaking from individual colonies was repeated until a pure culture was obtained. The pure culture was stored on nutrient agar slopes for identification purposes.

## ANAEROBIC INCUBATION

All samples taken were incubated anaerobically as well as aerobically; isolations made at random from the plate to detect obligate anaerobes. No obligate anaerobes were detected; all isolates examined proved to be facultative anaerobes. With this result no further tests were done except as outlined in Appendix F5.

### Method of Anaerobic incubation

A perspex glove box cabinet 1.2 m x 0.9 m x 0.6 m with a cylindrical entrance lock 0.3 m x 0.2 m diameter was used for anaerobic incubation. A gaseous environment of 85 percent Nitrogen, 10 percent Carbon Dioxide and 5 percent Hydrogen was used. Oxygen was purged from the environment by constantly circulating the gas through a container of granulated palladium coated catalyst followed by silica gel. The cabinet was maintained at 25$^0$C. After each transfer of petri dishes into or out of the cabinet three changes of the gas was made (the gas was bought ready mixed from New Zealand Industrial Gases) and extra Hydrogen was added to help speed the purging of any infiltrated oxygen.

Initially the cabinet was filled with the chosen gases in the following manner. The special gas mix plus extra hydrogen was injected until the rubber gloves were over inflated. This was than pumped out by vacuum pump until a negative pressure of approximately 10 kPa existed. More special gas was then injected and this whole cycle repeated six times. The cabinet was then left over-night with a surplus of hydrogen in the gas mix. A G.L.C. analysis of the gas was then made to check that no oxygen was present and to determine if the composition of gases was correct. Once this had been established a routine system of adding methylene blue indicator strips (BBC Disposable Anaerobic Indicator) whenever plates were added to the cabinet. At intervals another G.L.C. check was run to redetermine gas levels.

APPENDIX F2

BACTERIAL SAMPLE OF LOGPILE AT BALMORAL

| Pile | Height | Depth | Raw Data (counts per 20 $mm^3$) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | A | a | 0 | 0 | 0 | 3 | 52 | 0 | 32 | 2000 | | | | | |
| | | b | 0 | 0 | 0 | 1 | 0 | 0 | 31 | 0 | | | | | |
| | | c | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | | | | | |
| | B | a | 2000 | 2000 | 59 | 4 | 410 | 0 | 0 | 0 | | | | | |
| | | b | 0 | 35 | 1 | 0 | 19 | 0 | 0 | 0 | | | | | |
| | | c | 0 | 2000 | 1 | 0 | 6 | 0 | 0 | 0 | | | | | |
| | C | a | 30 | 100 | 500 | 3 | | | | | | | | | |
| | | b | 1 | 0 | 100 | 0 | | | | | | | | | |
| II | A | a | 34 | 1 | 0 | 4 | 1 | 10 | 2000 | | | | | | |
| | | b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | |
| | | c | 0 | 0 | 4 | 0 | 0 | 0 | 0 | | | | | | |
| | B | a | 23 | 0 | 0 | 27 | 5 | 0 | 4 | 100 | 0 | 9 | 0 | 0 | 12 | 0 |
| | | b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| | | c | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 1 |
| | C | a | 0 | 0 | 1 | 58 | 0 | 0 | 0 | 800 | 0 | | | | | |
| | | b | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 8 | 0 | | | | | |
| | | c | 1400 | 800 | 0 | 102 | 1 | 0 | 210 | 0 | 0 | | | | | |

APPENDIX F2 (continued)

| Pile | Height | Depth | Raw Data (counts per 20 $mm^3$) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| III | A | a | 0 | 1 | 78 | 0 | 0 | 0 | 0 | | | | | | | |
| | | b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| | | c | 1 | 2000 | 0 | 0 | 0 | 0 | 0 | | | | | | | |
| | B | a | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | | | |
| | | b | 250 | 18 | 14 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | | | |
| | | c | 400 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2000 | 0 | 0 | | | |
| | C | a | 0 | 700 | 1050 | 2000 | 78 | 300 | 0 | 560 | 0 | 5 | 4 | 0 | | |
| | | b | 0 | 126 | 1430 | 4 | 0 | 0 | 0 | 0 | 4 | 100 | 0 | 2000 | | |
| | | c | 0 | 150 | 760 | 240 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | | |
| IV | A | a | 60 | 0 | 9 | 100 | 1 | 180 | 0 | 0 | 0 | 16 | | | | |
| | | b | 2000 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 100 | | | | |
| | | c | 0 | 0 | 2000 | 0 | 0 | 0 | 1 | 0 | 0 | 2000 | | | | |
| | B | a | 0 | 0 | 2000 | 4 | 0 | 8 | 0 | 1 | 0 | 0 | 0 | 5 | 2000 | 0 |
| | | b | 0 | 1 | 0 | 2000 | 2000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | c | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2000 | 0 | 0 | 0 | 0 | 0 | 0 |
| | C | a | 0 | 0 | 23 | 0 | 0 | 0 | | | | | | | | |
| | | b | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | |
| | | c | 0 | 0 | 2000 | 1 | 0 | 0 | | | | | | | | |

APPENDIX F3

BACTERIAL SAMPLE OF LOGPILE AT BALMORAL

Analysis of Variance

| | df | ss | ms | F | |
|---|---|---|---|---|---|
| Variation among piles | 3 | 991808 | 330602.66 | 0.635 | ns |
| Heights within piles | 8 | 1703838 | 212979.75 | 1.054 | ns |
| Depths core within height | 24 | 4826208 | 201091.95 | 0.893 | ns |
| Depth core error | 294 | 66269203 | 225405.45 | | |
| Total | 329 | 73791054 | | | |

Expected Mean square

Variation among piles $= S^2 + 9.8S^2_{C<B} + 29.5S^2_{B<A} + 81.8S^2_A$

Heights within piles $= S^2 + 8.8S^2_{C<B} + 26.4S^2_{B<A}$

Depths core within height $= S^2 + 9.2S^2_{C<B}$

Depth core error $= S^2$

Therefore

$S^2 = 225405.45 = 96.46$ percent

$S^2_{C<B} = -2652.574 = -1.13$ percent

$S^2_{C<A} = 10916.951 = 4.67$ percent

Gas Liquid Chromatography

Varian series 1520 gas chromatograph

Column Poropak Q 100-80 mesh

Molecular Sieve No. 3A

Carrier gas Helium 455 kPa

Argon 455 kPa

Sample Column 60ml $min^{-1}$

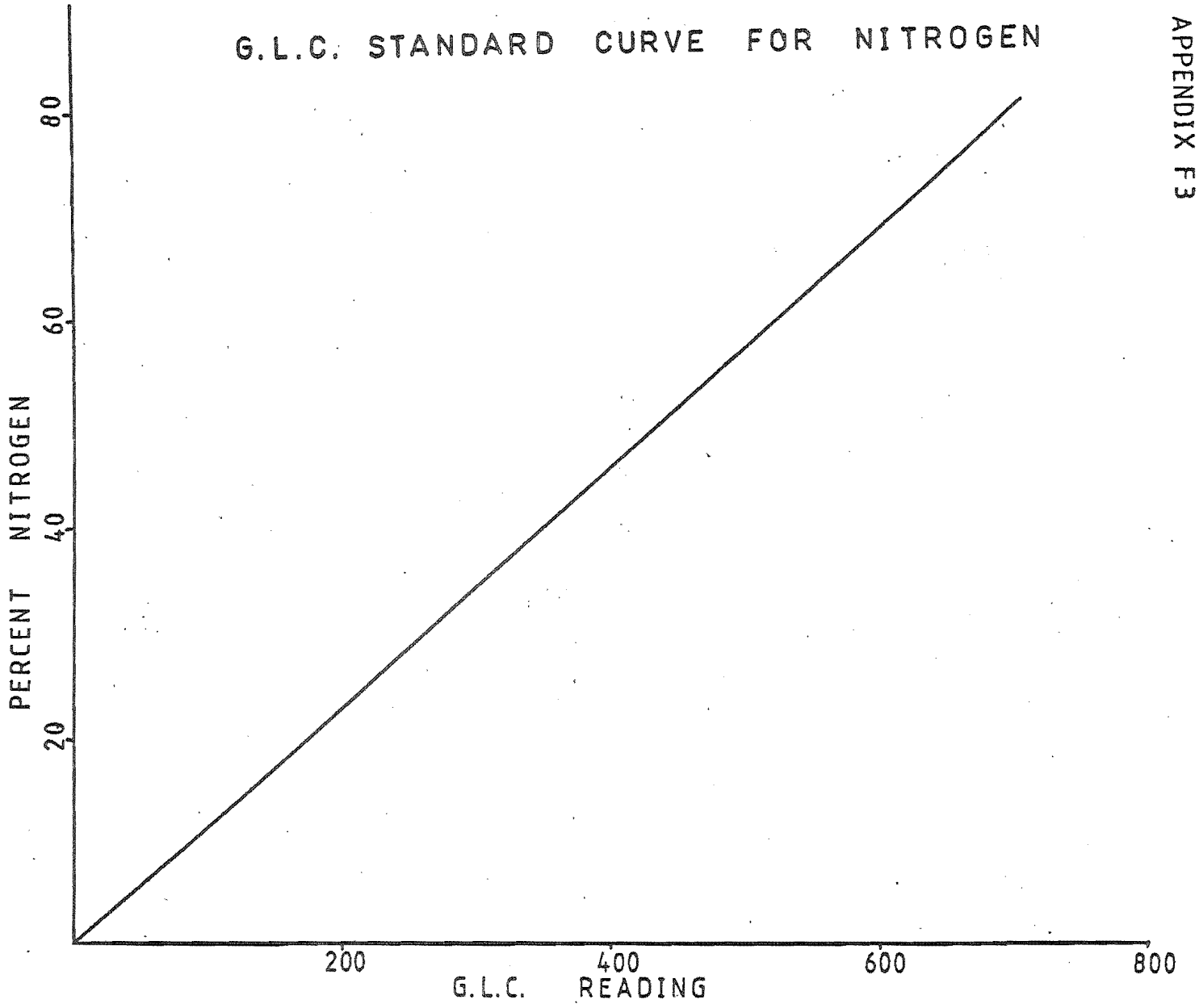
Reference Column 30ml $min^{-1}$

Temperature Injection Port 175°C

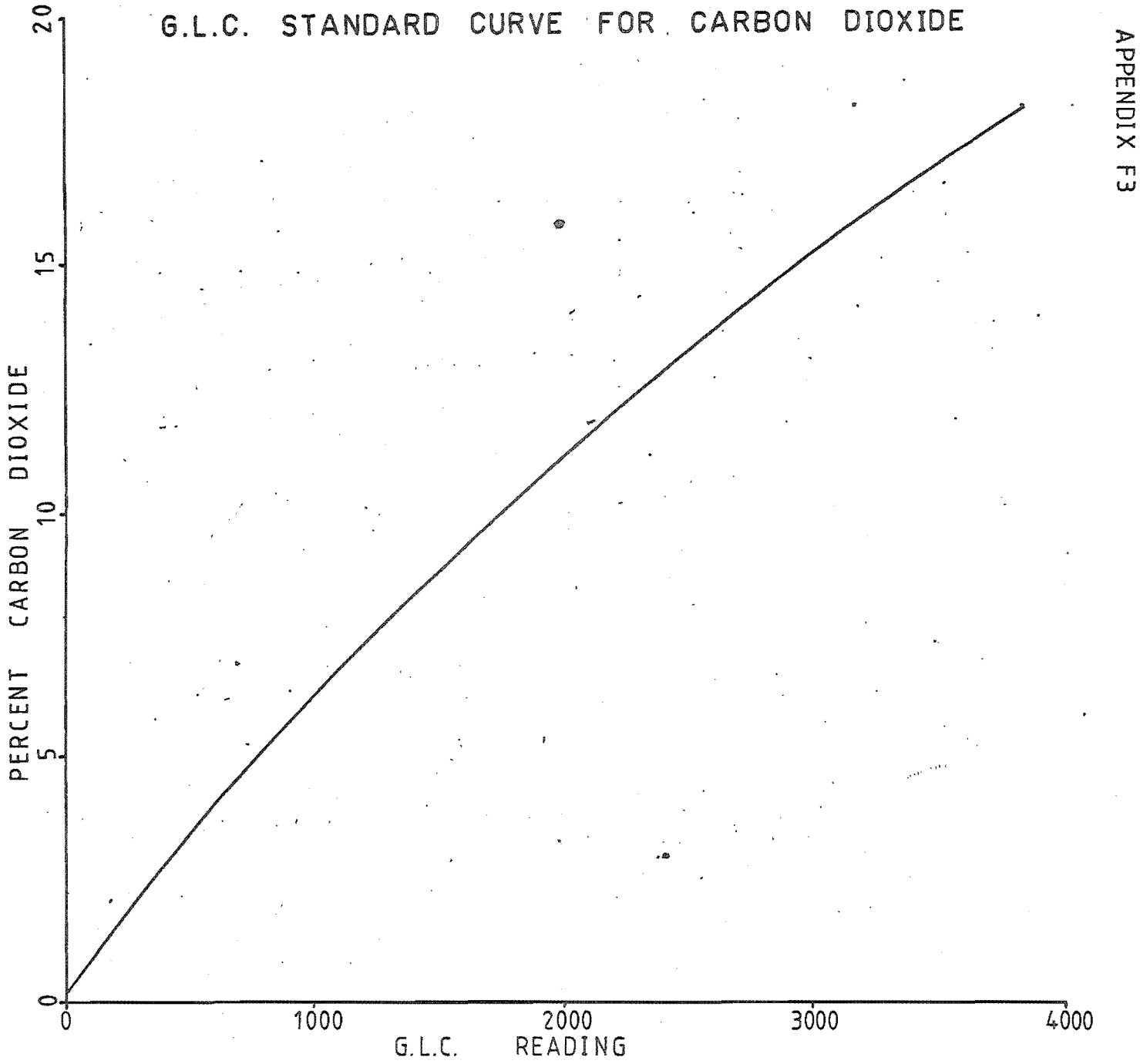
Detector 160°C

Oven 40°C

APPENDIX F3

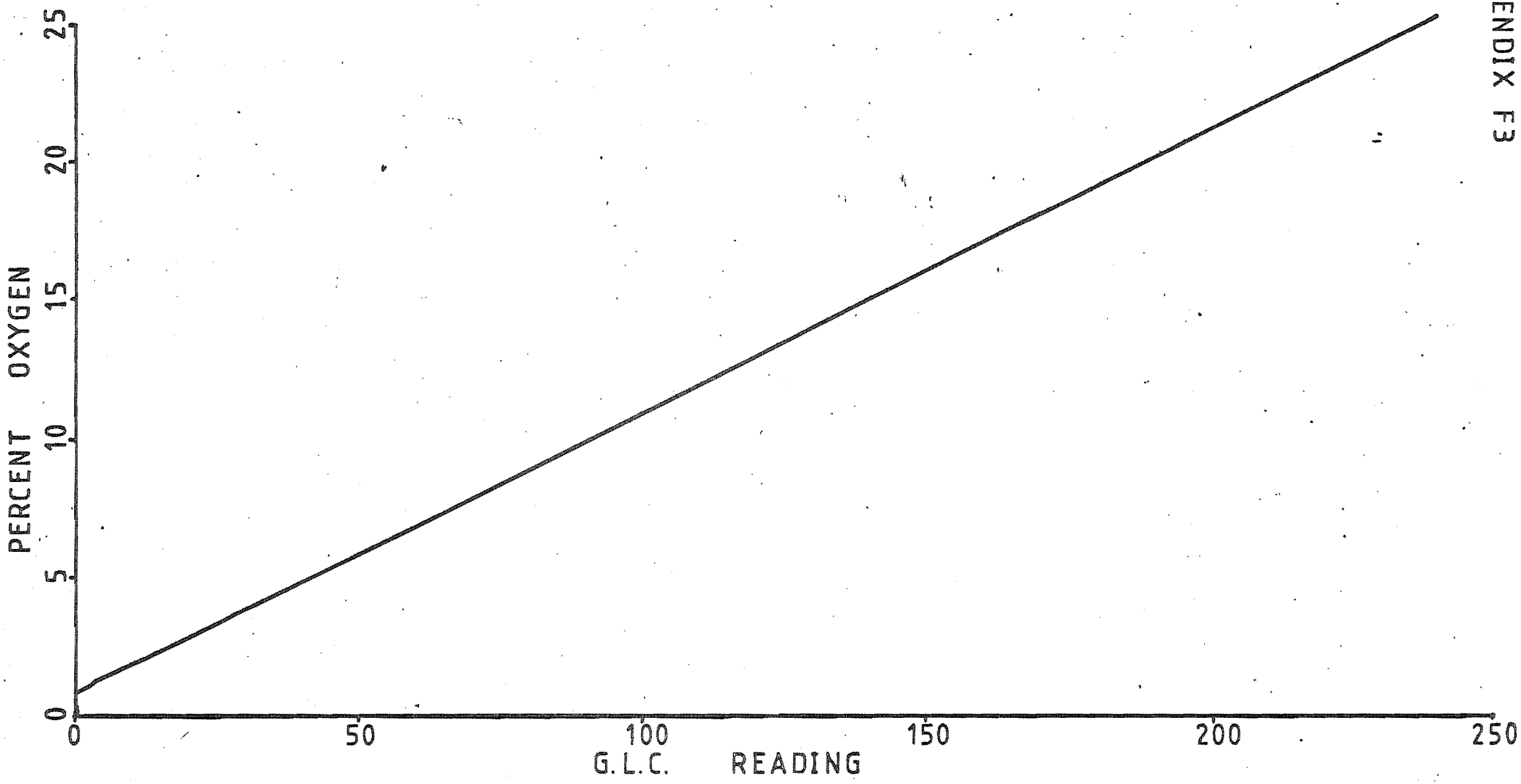

G.L.C. STANDARD CURVE FOR NITROGEN
PERCENT NITROGEN
20
40
60
80
200
400
600
800
G.L.C. READING

APPENDIX F3


G.L.C. STANDARD CURVE FOR CARBON DIOXIDE
PERCENT CARBON DIOXIDE
0
5
10
15
20
0
1000
2000
3000
4000
G.L.C. READING

APPENDIX F3

# G.L.C. STANDARD CURVE FOR OXYGEN



APPENDIX F4

FUNGI ISOLATED FROM SMALL LOGPILE

| Week | Unsprinkled Pile | Sprinkled Pile |
|---|---|---|
| 1-3 | 0 | 0 |
| 4 | Alternaria | 0 |
| 5-9 | 0 | 0 |
| 10 | Fusarium, Mycelia sterilia | 0 |
| 11 | Chaetomium | 0 |
| 12 | Fusarium, Alternaria | 0 |
| 13 | Alternaria | Alternaria |
| 14 | Polystictus, Trichoderma, Fusarium | Fusarium, Alternaria |
| 15 | Alternaria, Fusarium, Trichoderma | Fusarium |
| 17 | Fusarium | Chaetomium |
| 19 | Fusarium, Trichoderma | Fusarium |
| 21 | Fusarium | 0 |
| 23 | Fusarium, Alternaria, Verticillium | 0 |
| 27 | Fusarium, Verticillium | 0 |

APPENDIX F5

# TESTS USED TO CHARACTERISE BACTERIA

All incubation was at 25$^{0}$C. Isolates were not always examined by the full range of tests. All Minitek (BBL) discs were incubated in special trays for 18 hours only.

## F5:1 MORPHOLOGICAL CHARACTERS

Cell Morphology: Wet mounts were prepared from young (24 hours) and older cultures (70-76 hours) on nutrient agar and examined by phase contrast microscopy.

Motility: Motility was recorded during the phase contrast examination of both wet mounts. If either showed motility the isolate was regarded as positive for this character.

Gram Reaction: Air dried smears of 20-28 hour old cultures were fixed and stained as outlined by Skerman (1967). If the result was doubtful it was repeated. All cultures were also stained and examined when 70-76 hours old.

## F5:2 CULTURAL CHARACTERS

Colour: This was routinely observed on nutrient agar.

## F5:3 UTILISATION OF CARBON COMPOUNDS

Utilisation of Glucose: This was determined by using glucose impregnated discs (Minitek, BBL). Fermentative

determination was achieved by oil overlay.

Acid from Carbon Sources: Arabinose, mannitol, starch and sucrose were tested by the use of impregnated discs (Minitek, BBL). Phenol red was the indicator used to determine acid production.

Utilisation of Cellulose and Pectin: These were determined by the inclusion of the appropriate carbon source into a nutrient salt solution as detailed:

| | | |
|---|---|---|
| $KH_2PO_4$ | 0.1 | g |
| $K_2HPO_4$ | 0.75 | g |
| NaCl | 0.25 | g |
| $(NH_4)_2SO_4$ | 1.25 | g |
| $MgSO_4.7H_2O$ | 0.1 | g |
| $FeSO_4.7H_2O$ | 0.002 | g |
| agar | 15.0 | g |
| water to | 1.0 | ℓ |
| Cellulose or Pectin | 20.0 | g |

(Crawford et al 1973)

Voges Proskauer Test: This was determined by the use of an impregnated disc (Minitek, BBL). After incubation, colour was developed by adding a drop each of 40 percent potassium hydroxide then 5 percent 1-naphthol, a red colour indicating positive.

F5:4 BIOCHEMICAL TESTS

Catalase Test: A loopful of growth from a nutrient agar plate was emulsified in a drop of 10 volume $H_2O_2$ on a clean glass slide and examined for the production of gas bubbles.

Oxidase Test: Kovac's method, as modified by Steel (1961) was used.

Reduction of Nitrate: This was determined by the use of an impregnated disc (Minitek, BBL). Colour development was by the addition of a drop of a solution of: 8 g sulfanilic acid + 5 N acetic acid to 1 ℓ, followed by a drop of a solution of: 6 ml N, N-dimethyl-1-naphthalamine + 5 N acetic acid to 1 ℓ.

Indole/$H_2S$ production: This was determined by the use of an impregnated disc (Minitek, BBL). Any black colouring around the disc was noted as evidence of $H_2S$ production.

Urease Production: This was determined by the use of an impregnated disc (Minitek, BBL).

Starch Hydrolysis: The same salts medium as employed for cellulose and pectin was employed with the addition of 2 percent starch. Plates were inoculated by streaking and hydrolysis detected after five days' growth by flooding the surface of the agar with Lugol's iodine solution.

F5:5 ANAEROBIC GROWTH

All samples taken were spread onto cooked meat medium (BBL) with the addition of 1 percent agar and incubated in the glove box described earlier. Counts were taken and random isolated were compared to isolates of the same sample grown aerobically.

SELECTION FOR TESTING

All isolates obtained from the method outlined in F1 were gram stained and checked visually. Those isolates showing differences in any or all the characters of colony morphology, rate of growth, gram reaction and visual assessment by water mount were further tested by the methods outlined in F5.

APPENDIX F6

AVERAGE COUNT OF BACTERIA FROM EACH CORE TAKEN FROM SMALL LOGPILE

| Week | Unsprinkled | | | | | Sprinkled | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1000 | 1000 | 1000 | 12 | 4 |
| 4 | 1 | 1 | 1 | 1 | 1 | 1000 | 1000 | 1000 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 1 | 1 | 1000 | 1000 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 6 | 1000 | 1 | 2 | 0 | 1 | 1000 | 3 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 7 | 1 | 1 | 0 | 1 | 0 | 1000 | 1000 | 1000 | 500 | 1 | 1 | 0 | 0 | 0 | 0 |
| 8 | 1 | 0 | 0 | 0 | 0 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 1000 | 150 | 30 | 6 | 5 | 5 | 1 | 1 | 0 | 0 |
| 10 | 1000 | 0 | 0 | 0 | 0 | 1000 | 1000 | 1000 | 1000 | 1000 | 500 | 10 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 | 1000 | 1000 | 1000 | 1000 | 700 | 0 | 0 | 0 | 0 | 0 |
| 12 | 1000 | 1000 | 1000 | 50 | 0 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 150 | 3 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 1000 | 1000 | 1000 | 1000 | 10 | 1 | 1 | 1 | 1 | 0 |
| 14 | 1000 | 1000 | 6 | 5 | 0 | 1000 | 1000 | 1000 | 1000 | 10 | 1 | 1 | 1 | 1 | 1 |
| 15 | 1000 | 300 | 300 | 0 | 0 | 1000 | 1000 | 1000 | 1000 | 1000 | 50 | 0 | 0 | 0 | 0 |
| 17 | 5200 | 3800 | 2800 | 0 | 0 | 110 | 100 | 16 | 6 | 4 | 1 | 1 | 0 | 0 | 0 |
| 19 | 3900 | 3100 | 2800 | 2600 | 2300 | 5100 | 4100 | 3100 | 2100 | 310 | 210 | 110 | 42 | 41 | 1 |
| 21 | 9000 | 9000 | 3000 | 500 | 0 | 9200 | 2200 | 4500 | 500 | 30 | 30 | 5 | 0 | 0 | 0 |
| 27 | 1100 | 2 | 1 | 0 | 0 | 9200 | 3200 | 2400 | 1200 | 800 | 100 | 0 | 0 | 0 | 0 |

APPENDIX G1

## CALCULATION OF THEORETICAL GAS VOLUME IN WOOD

Percent void volume = wood volume - volume occupied by wood substance - volume water

Wood substance volume = dry mass/1.5 (density wood substance = 1.5 g/ml = volume x dry density/1.5)

and volume water = wet weight - dry weight

so percent void volume = wood volume - (volume x dry density/1.5) - (wet wt - dry wt)

Therefore

Gas volume = percent void volume x void space

= percent void volume x volume (1-dry density/1.5)

For Sapwood

gas volume = 0.08 x 441.78(1-0.43/1.5)

= 25.21 ml

For Heartwood

gas volume = 0.60 x 441.78(1-0.35/1.5)

= 203.22 ml

APPENDIX G2

## ANALYSIS OF GAS SAMPLES TAKEN FROM SPRINKLED BALMORAL WOOD

Oxygen ANOVA

| | df | ss | ms | F |
|---|---|---|---|---|
| Group | 1 | 97.524 | 97.52 | 2.28 |
| Error | 18 | 769.345 | 42.74 | |
| Total | | 866.869 | | |

Carbon Dioxide ANOVA

| | df | ss | ms | F |
|---|---|---|---|---|
| Group | 1 | 112.006 | 112.006 | 4.23 |
| Error | 18 | 476.331 | 26.463 | |
| Total | | 588.337 | | |

Nitrogen ANOVA

| | df | ss | ms | F |
|---|---|---|---|---|
| Group | 1 | 53.379 | 53.379 | 0.28 |
| Error | 18 | 2948.56 | 184.285 | |
| Total | | 3001.94 | | |

APPENDIX G4

ANOVA Effect of Temperatures on Decay Capacity of Two Wood Rotting Fungi

| Source | df | ss | ms | F |
|---|---|---|---|---|
| Subgroups | 11 | 0.31089 | 0.02826 | |
| treatments | 3 | 0.06396 | 0.02132 | 7.06 ** |
| temperature | 2 | 0.19585 | 0.09792 | 32.42 ** |
| interaction | 6 | 0.05108 | $8.513 \times 10^{-3}$ | 2.82 ns |
| Error | 84 | 0.25368 | $3.020 \times 10^{-3}$ | |

| Source | df | ss | ms | F |
|---|---|---|---|---|
| Subgroups | 5 | 0.22237 | 0.04447 | |
| bacteria | 1 | 0.01915 | 0.01915 | 5.04 * |
| temperature | 2 | 0.19584 | 0.09792 | 25.75 ** |
| interaction | 2 | $7.378 \times 10^{-3}$ | $3.689 \times 10^{-3}$ | 0.97 ns |
| Error | 90 | 0.34220 | $3.802 \times 10^{-3}$ | |

| Source | df | ss | ms | F |
|---|---|---|---|---|
| Subgroups | 5 | 0.27939 | 0.05587 | |
| fungi | 1 | 0.04309 | 0.04309 | 13.60 ** |
| temperature | 2 | 0.19584 | 0.09792 | 30.90 ** |
| interaction | 2 | 0.04044 | 0.02022 | 6.38 ** |
| Error | 90 | 0.28518 | $3.168 \times 10^{-3}$ | |

** significant at 1 percent

* significant at 5 percent

ns not significant

APPENDIX G4 (continued)

Weight Losses Caused by Wood Rotting Fungi

| | |
|---|---|
| a x b | F = 0.27 ns |
| c x d | F = 0.08 ns |
| e x f | F = 0.18 ns |
| ab x cd | F = 0.37 ns |
| ab x ef | F = 0.75 ns |
| g x h | F = 0.32 ns |
| i x j | F = 2.60 ns |
| k x l | F = 1.73 ns |
| gh x ij | F = 1.97 ns |
| h x j | F = 7.95 significant at 1 percent |
| h x l | F = 155.62 significant at 1 percent |

Weight Changes in Wetwood inoculated with Bacteria and *Coniophora puteana*

ANOVA

| | df | ss | ms | F |
|---|---|---|---|---|
| Treatments | 4 | 0.10738 | 0.02684 | 226.95 ** |
| Error | 15 | 0.00165 | $1.183 \times 10^{-4}$ | |
| Total | 19 | 0.10904 | | |

Test for equal sample variances $H_0 : s_1^2 = s_2^2$

| | | | |
|---|---|---|---|
| a x b | F = 53.194 ** | b x d | F = 4.28 ns |
| a x c | F = 59.598 ** | b x e | F = 5.58 ns |
| a x d | F = 227.799 ** | c x d | F = 3.82 ns |
| a x e | F = 297.171 ** | c x e | F = 4.98 ns |
| | | d x e | F = 1.31 ns |

** significant at 1 percent ns not significant